UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS E TECNOLOGIA

ONTOGENY OF BEHAVIOURAL ABILITIES IN TEMPERATE REEF FISH LARVAE

(Doutoramento em Ciências do Mar, especialidade de Ecologia Marinha)

ANA MARGARIDA DA SILVA FARIA

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ONTOGENIA DAS CAPACIDADES COMPORTAMENTAIS EM PEIXES DE RECIFES TEMPERADOS

Resumo

A maior parte das espécies associadas a recifes possui uma fase larvar pelágica, seguida de uma fase juvenil e adulta demersal. A fase pelágica pode apresentar consideráveis capacidades de dispersão. A visão tradicional, baseada em estudos de um conjunto limitado de espécies de sistemas temperados, pressupunha que as capacidades natatórias das larvas no plano horizontal eram muito limitadas e, dessa forma, irrelevantes para o potencial de dispersão. Segundo esta perspectiva, a dispersão era essencialmente explicada por fenómenos de transporte passivo e a única informação necessária para modelar a dispersão seriam as correntes e a duração da fase larvar. No entanto, nas últimas duas décadas, a investigação do comportamento natatório das larvas de recifes tropicais veio provar que as larvas estão longe de serem partículas passivas, e têm capacidades comportamentais consideráveis, capazes de influenciar os seus padrões de dispersão.

Apesar dos recentes avanços nos estudos de comportamento larvar em peixes de recifes tropicais, os estudos de comportamento em peixes temperados são ainda escassos. Nesse sentido, esta tese representa um significativo contributo para o estudo das capacidades natatórias de peixes de sistemas temperados. A ontogenia do comportamento natatório foi investigada em quatro espécies temperadas (2 Gobiesocidae, 1 Sparidae, 1 Soleidae) e uma espécie temperada-quente (Sciaenidae). Os resultados sugerem que a diferença de capacidades natatórias entre espécies tropicais e temperadas não é significativa quando aspectos taxonómicos, morfológicos e estados de desenvolvimento são tidos em conta. Adicionalmente, foi analisada a influência da condição nutricional no comportamento

natatório e observou-se que larvas em inanição são capazes de levar a cabo comportamentos de fuga a predadores e captura de presas, mas em termos de potencial de dispersão, estas larvas são significativamente afectadas, compromentendo assim a sua sobrevivência e futuro recrutamento.

Palavras-chave: comportamento, capacidade natatória, ontogenia, larvas de peixes costeiros, recifes temperados, recifes tropicais, condição nutricional.

ONTOGENY OF BEHAVIOURAL ABILITIES IN TEMPERATE REEF FISH LARVAE

Abstract

Most reef associated fish species have a pelagic larval phase, followed by a more sedentary juvenile and adult stage. During the pelagic phase, larvae have the potential to disperse over a wide area. Traditionally, and based on research on swimming performance of a limited suite of temperate marine larval fishes, it was thought that swimming abilities of larval fishes in the horizontal plane were very limited and were largely irrelevant to issues of dispersal. With this perspective, larval dispersal was presumed to be governed by hydrographic advection and to model dispersal one simply needed to have information on currents and pelagic larval duration. However, in the last two decades, research on swimming behaviour of tropical reef fish larvae have shown that larvae are far from passive particles, and have considerable influence over their trajectories during the pelagic phase.

Despite the recent advances on larval behaviour in coral reef environments, studies on behaviour of temperate fish larvae are still scarce. In this sense, the present work represents a major contribution to the implications of larval behaviour in temperate ecosystems. The ontogeny of larval swimming behaviour was evaluated in four temperate species (two gobiedocids, one sparid and one soleid) and one warm-temperate species (sciaenid). Overall, results suggest that there isn't a strong difference in swimming speeds between temperate and tropical fish larvae when taxonomy, morphology and developmental stage are taken in consideration. Moreover, the influence of nutritional condition on swimming performance was evaluated and data revealed that even deprived of food, larvae may be able of performing escaping and foraging behaviours, but sub-lethal effects of starvation may affect dispersal potential by greatly reducing endurance

swimming, and therefore compromise subsequent survival and recruitment to the adult population.

Key-words: behaviour, swimming abilities, ontogeny, reef fish larvae, temperate reefs, tropical reefs, nutritional condition

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CHAPTER I. GENERAL INTRODUCTION

1. Why Study Fish Larvae?

Fisheries scientists and managers are not only concerned about adult populations of fishes, but also about growth and survival during the earliest life stages as variability in those processes can lead to 10-fold or greater differences in numbers of recruits that survive to catchable size (Houde 2001, 2002). Thus, determining the causes and consequences of variability in recruitment has become a central problem in marine ecology and fisheries biology. Recruitment, in the broadest sense, refers to the addition of new individuals from the pelagic habitat to the adult habitat measured after larval settlement. It represents the end product of a series of dynamic physical and biological processes that vary spatially and temporally, and it's their combination that determines recruitment success.

The need to understand the processes underlying the fluctuations in fished populations led to the development of several hypotheses regarding their primary causes. Hjort (1914) was the first to explicitly link feeding, larval survival and subsequent recruitment to food abundance during the transition of larvae from endogenous (yolk) to exogenous (plankton) feeding. The so-called "critical period" hypothesis, proposed that incidence of starvation at the time of yolk-sac exhaustion, when larvae first required plankton as food, was the primary factor determining variability in year-class recruitment success (Houde 2001).

Almost 60 years following Hjort's publication, Cushing proposed the "match-mismatch" hypothesis, which emphasized the importance of temporal coincidence in spawning and bloom dynamics of plankton, the primary food of larval fish. A "match" between spawning and spring plankton blooms ensures larval growth and survival, while a "mismatch" results in high mortality (Houde 2001). While the mechanism of larval

mortality is again starvation, the "match-mismatch" hypothesis removed the restriction that food-mediated mortality leading to recruitment variability is limited to a particular critical development period. Rather, food limitation during any part of the larval period could be a major contributor to recruitment variability, and abiotic factors that regulate water-column destratification and the timing and intensity of seasonal production cycles may be involved (Cowen and Shaw 2002).

The "stable ocean" hypothesis, proposed by Lasker in the 1970s, is another nutrition-related explanation for variability in larval survival. Lasker accepted the initial premise that food for first feeding larvae may be limited, but suggested that there are times and places in the sea where food aggregations occur, upon which larval fish survival depends (Cowen and Shaw 2002). He hypothesized that relaxation of storm winds and intense upwelling results in a stable, vertically stratified ocean in which strata of fish larvae and their prey coincide, promoting larval nutrition and survival (Houde 2001).

Models of recruitment have also integrated physical factors. As an example, Iles and Sinclair (1982) proposed an idea that has been referred to as the "Larval Retention" or "Member/Vagrant" hypothesis. This hypothesis emphasizes physics and circulation features, rather than nutritional factors, as the controller of recruitment variability. It states that, through the years, species tend to spawn at specific times and places within predictable and distinct circulation features. This enhances the retention of its "members" within these features by limiting dispersal of passive eggs and weakly swimming larvae until they are able to develop the ability to control their own distribution. In this way, retention helps to maintain the population's distinct geographic distribution. In fact, they proposed that population size can also be regulated in this way. A density-dependent mechanism may be activated when the population size increases to the point that a portion of the expanding spawning population is displaced to less favorable spawning grounds.

These less favorable areas have a lower capability to retain larvae near the spawning and nursery grounds because residence times within the circulation feature are shorter. This eventually will lead to individuals being lost from the local spawning population ("vagrants"). The result of vagrancy, therefore, is to increase the loss rate of eggs and larvae as the size of the spawning stock increases and spawning grounds expand outside of the population's favorable retention area (Cowen and Shaw 2002).

Other models of recruitment strength have integrated growth and mortality, representing a different way of thinking about the recruitment problem. It eventually became increasingly clear that predation is a major source of egg and larval mortality in fishes, and therefore a potent regulator of year-class size (Houde 2002). Because most fish larvae die soon after hatching and predation is now believed to be a major source of larval mortality, it follows that survivors from cohorts exposed to predators may be exceptional individuals with respect to characteristics that shape predation vulnerability. The "Bigger is Better" hypothesis states that individuals that grow faster and become larger take advantage of an enhanced ability to avoid predators and acquire food (Miller et al. 1988, Cowen and Sponaugle 1997, Cowen and Shaw 2002). The "Stage Duration" hypothesis defends that given that mortality is highest in the beginning of the pelagic period, the faster the development, the greater will be the chance of surviving (Blaxter 1986, Chambers and Legget 1987, Houde 1987). Despite the evidences that larger and/or faster growing members of a cohort have a survival advantage over smaller conspecifics, a number of examples indicate non-selective mortality with no obvious size advantages (e.g. Litvak and Leggett 1992, Bertram and Leggett 1994, Pepin and Shears 1995).

More recently, recruitment models tend to incorporate several aspects of the interaction between larvae and the environment. A recent hypothesis receiving considerable interest is the "Ocean Triad" hypothesis (Bakun 1996), in which enrichment (e.g. upwelling),

concentration mechanisms (e.g. convergences and fronts), and transport-and-retention processes are key factors underlying recruitment variability in marine fish populations.

Considering the above, it should be clear that larval fish distribution, abundance and survival is controlled by abiotic (e.g. temperature, salinity, turbidity, water current speeds and directions), and biotic factors (e.g. adult spawning condition, behaviour and abundance, food availability, potential predators, larval condition and behaviour) and it is the dynamic interaction among these factors that determines recruitment success.

1.1 Recruitment, Larval Supply and Dynamics of Reef Fish Populations

Historically, fisheries studies provided a major contribution to our understanding of the dynamics of marine species (e.g. Houde 1987, Bailey and Houde 1989, Fogarty et al. 1991), leading to recognition of the great variability that exists in pelagic fish recruitment. Nevertheless, these studies typically refer to exploitable species that are pelagic for their entire life cycle, in contrast to reef fishes, which have a bipartite life history (Leis 1991), divided between the benthic adult phase, living closely attached to the reef, and the pelagic larval phase, whose individuals live in open water, in some cases drifting hundreds of kilometers away from any reef. For many years, coral reef fish assemblages were considered stable and regulated by density dependant mechanisms (Doherty and Williams 1988). However, recent studies of reef-fish assemblages have proved that patterns of recruitment are often highly variable in both time and space (Doherty and Williams 1988, Caley et al. 1996, Sale 2004). Determining the causes and consequences of variability in recruitment has, then, become a central problem in marine ecology and fisheries biology. Reef fish ecologists have focused on the hypothesis that variation in larval supply is a major cause of recruitment variability (see Reviews by Doherty 1987, Caley et al. 1996, Sale 2004). Larval supply may vary based on biological parameters,

such as egg production (Meekan et al. 1993), early life history traits (Cowen and Sponaugle 1997, Raventós 2005, Sponaugle et al. 2006), lunar and tidal cues (Sponaugle and Cowen 1996), among others; supply may also be influenced by stochastic factors, such as larval growth, condition and mortality (Houde 1997, Cowen et al. 2000), and various oceanographic processes, such as currents and tides (e.g. Sponaugle and Cowen 1996), eddies (e.g. Sponaugle et al. 2005, D'Alessandro et al. 2007), winds, among others. Other than larval supply, patterns of recruitment are also likely to be strongly influenced by ecological processes after and/or during settlement (transition from the pelagic to the benthic habitat), such as predation (Carr and Hixon 1995), competition (Bonin et al. 2009), behaviour (Huebert and Sponaugle 2009) and habitat selection (Risk 1997). Therefore, both pre- and post-settlement processes can be important sources of variation in population replenishment. Still, the extent to which populations are more influenced by larval supply or other factors occurring during or after settlement remains controversial.

Links between larval supply and recruitment have been demonstrated for some species. Milicich et al. (1992) showed good correlation between larval survival and early recruitment levels in three coral-reef species of Pomacentridae, suggesting that pre-settlement distributions may be the major determinant of early recruitment patterns despite mediating influences from factors such as habitat selection and post-settlement mortality. More recently, Grorud-Colvert and Sponaugle (2009) found that, for at least the first month post-settlement, larval supply is a reasonable predictor of recruitment for the coral-reef damselfish *Stegastes partitus*. On a temperate environment, Hamer and Jenkins (1996) sampled pre-settlement larvae and post-settlement recruits of a demersal species, *Sillaginoides punctata*, and also found a positive correlation between larval

supply and post-settlement recruits. Nevertheless, these authors highlight the possibility of post-settlement processes influencing recruitment at longer time scales.

Contrary to the hypothesis that variability in larval supply is the primary factor influencing year-class strength, other studies have found that post-settlement processes seem more important in determining recruitment. Sponaugle and Cowen (1996) examined the temporal and spatial patterns of supply and recruitment of two reef fishes, a Pomacentridae and an Acanthuridae, and found that for the former species, spatial and temporal patterns of recruitment were related to larval supply. In contrast, benthic population densities of juvenile *A. bahianus* (Acanthuridae) were directly the opposite of patterns of larval supply, suggesting that for some species, post-settlement processes such as habitat selection may be more influential in creating spatial patterns of recruitment. Levin (1996) determined the importance of larval supply to explain spatial patterns of larval settlement and recruitment for a common Gulf of Maine fish and concluded that at a scale of hundreds of meters to kilometers, processes occurring at or within the first 24 h of settlement were influencing recruitment. Steele et al. (2002) rejected the hypothesis that spatial patterns in recruitment of kelp bass (*Paralabrax clathratus*) were set by larval supply. Recruitment was strongly correlated with the density of 1 yr old kelp bass. Recently, Crean et al. (2009) investigated the potential influence of endemism on the relationship between larval supply and recruitment in reef fish populations at Lord Howe Island, Australia. They found that recruitment was correlated with larval supply in endemics; the two phenomena were not correlated in non-endemics, likely due to a combination of low larval supply and low post-settlement survival of non-endemics.

It can thus be concluded that recruitment is highly variable, in time and space, and patterns of recruitment may vary between species and places, and both pre-settlement and post-settlement processes may act together or individually to influence recruitment.

1.2 Larval Dispersal, Retention and Connectivity of Reef Fish Populations

The embryonic and larval periods have important ecological and evolutionary functions, and for most demersal marine fishes, they represent an effective means of dispersal that can extend the range of a population and mix the gene pool (Cowen 2002, Kinlan and Gaines 2003, Sale 2004). Traditionally, based on limited evidence, it was assumed that larval dispersal was largely passive, with propagules being transported by currents. In this view, the extent of larval dispersal was believed as solely dependent on patterns of water movement and duration of larval life. This "simplifying assumption" (Leis 2002) assumed that larval behaviour had little influence on dispersal potential and it was used by modelers to predict pelagic dispersal (e.g. Frank et al. 1993, Roberts 1997), and by fishery and conservation managers to set geographic boundaries and scales of management (Leis 2006). From this perspective, local populations were expected to be replenished largely by larvae derived from elsewhere (Williams et al. 1984, Roberts 1997), functioning as *open* populations, connected by the larval stage. This simplifying assumption suggests that populations of marine, demersal fishes in different locations may be connected by dispersal between them, and the extent to which these populations are linked is termed *connectivity* (movement of individuals within and among local or sub-populations; Cowen and Sponaugle 2009).

However, in the last two decades, research on the pre-settlement stages of reef fishes has revealed remarkable behavioural abilities that make the simplifying assumption and its components untenable (Leis 2006). Recent studies of the swimming abilities of larval coral reef fishes confirm that most are more capable swimmers than are the temperate species, that were the initial focus of studies of larval fish biology (such as plaice). Studies of sensory capabilities are likewise demonstrating previously unsuspected competencies of fish larvae (see Review by Myrberg and Fuiman 2002), and simple

behavioural experiments are suggesting that larval fish may use these sensory and swimming capabilities to swim towards reefs that they can hear or smell (Stobutzki and Bellwood 1998, Tolimieri et al. 2002, Leis and Carson-Ewart 2000a, Montgomery et al. 2001, 2006, Leis et al. 2003, Review by Leis and McCormick 2002), or to remain in the near vicinity of reefs, rather than being advected away from them (Doherty et al. 1996). Supporting field-based evidence of the importance of larval behaviour to dispersal or retention, models incorporating behavioural considerations markedly changed their predictions relative to conclusions drawn from models lacking behavioural parameters (Wolansky et al. 1997, Armsworth 2000, 2001, Armsworth et al. 2001, Irisson et al. 2004, Cowen et al. 2006). Only when models include directional swimming at realistic speeds do they reproduce field results.

The impressive behavioural capabilities of fish larvae do not imply that hydrography play an unimportant role in dispersal outcome (Cowen 2002, Sponaugle et al. 2002). The flow environment of most coastal oceans is determined by tidal, wind, seasonal and/or episodic (e.g., storm generated) variability which exists in both horizontal and vertical planes (Sponaugle et al. 2002, see Review by Cowen 2002). Flows in nearshore, shallow environments, are different from coastal and deep-ocean flows mainly because of the shoreline barrier, shallow depths, bathymetric features associated with the continental shelf, and nearshore inputs of freshwater (Pineda et al. 2007). Near to the coastal boundary, flows are weaker due to bed friction and/or the form drag effect of an indented coastline. This coastal boundary layer offers opportunities for retention (Graham and Largier 1997, see Review by Gawarkiewicz et al. 2007). In addition to slower flows nearshore, Largier (2003) discusses the importance of the proximity of the coastal boundary in limiting the horizontal scale of eddy motions, and thus limiting cross-shore dispersion. This is important because nearshore flows tend to be parallel to the coast with

limited advective transport in the cross-shore direction (Gawarkiewicz et al. 2007). Because currents are generally stronger further from the shore (away from boundary-layer effects), larvae that remain close to the shore will be advected shorter distances alongshore. Moreover, larvae also may be able to reduce dispersal by remaining in the benthic boundary layer (BBL) where flow is reduced to very low levels (Nowell and Jumars 1984). Among fishes, post-flexion or late-stage larvae of several temperate and tropical families have been found in or near the benthic boundary layer (Leis 1986, Breitburg 1991). These attributes of boundary layers have the potential to reduce alongshore larval transport, and may interact with behavioral responses, recirculation zones, and obstructing vegetation (seagrass beds, mangroves, kelp forests) to elevate local retention.

The debate "retention" *vs.* "dispersal" has become a central issue in reef ecology (Warner and Cowen 2002, Mora and Sale 2002) and its resolution has important implications for our understanding of the populations' structure and dynamics and, ultimately, management of marine populations. This discussion has led to the resurgence of interest in the question of connectivity and how open reef fish populations can be (Jones et al. 1999, Swearer et al. 1999, Cowen et al. 2000, Mora and Sale 2002, Jones et al. 2009). Recent studies suggest that many coral reef fish populations may be partly closed (Jones et al. 1999, Swearer et al. 1999, Cowen et al. 2000). This means that the populations can be self-recruited, *i.e.*, a significant proportion of juveniles successfully recruiting to a reef are the progeny of the resident adult population.

Many new technologies for estimating dispersal distances have emerged in the last decade, allowing a more accurate assessment of the extent to which reef fish populations are replenished by local or exogenous recruits (Reviews by Mora and Sale 2002, Cowen and Sponaugle 2009, Jones et al. 2009). Direct observations of larval behaviour (Leis and

Carson-Ewart 1999, 2002, Leis 2006, Leis et al. 2007, 2009a, b, Fisher et al. 2000), population genetics to track patterns and levels of migration (Shulman 1998, Planes 2002, Taylor and Hellberg 2003), otolith microchemistry (Swearer et al. 1999, Thorrold et al. 2007, Hamilton et al. 2008, Hamilton and Warner 2009, Ruttenberg et al. 2008), tagging studies using otoliths (Jones et al. 1999, 2005, Almany et al. 2007), and the application of genetic parentage analysis (Jones et al. 2005, Planes et al. 2009) are all approaches that have been used to measure connectivity of reef populations. However, one should bear in mind that each of these techniques has its own limitations and cannot be used alone to define temporal and spatial dispersal scales, and determine the extent of larval dispersal among populations. A multidisciplinary approach combining physical oceanography, larval behaviour, tagging studies, genetic analysis and sophisticated modeling techniques should be encouraged (Mora and Sale 2002).

The current concern for analyses of larval connectivity has been stimulated by the proliferation of coral reef Marine Protected Areas (MPAs; Mora et al. 2006), created to fulfill the purposes of biodiversity conservation and/or fishery replenishment. To be done properly, the establishment of protected areas, whether for conservation or fisheries applications, must be conducted in a context of detailed knowledge about the existing scale of demographic connectivity (Palumbi 2003, Sale 2004). At this point it is important to distinguish between demographic and genetic connectivity. Demographic connectivity is the movement of individuals between populations. On the other hand, genetic connectivity refers to the movement of genes between populations, and is expected to operate over larger geographic scales than demographic connectivity (Swearer et al. 2002, Palumbi 2003). Genetic connectivity is of evolutionary and biogeographic significance, whereas demographic connectivity is of ecological and management significance (Leis

2006) and is therefore, the most relevant for MPA design. The levels of connectivity among local populations will determine whether they function as essentially isolated, ‘‘almost closed’’ populations, or as a metapopulation, with the separate dynamics of individual populations being buffered by subsidy of recruitment from other populations (Sale 2004). Designing reserve networks (e.g., reserve location, size and spacing) that adequately protect connectivity requires an understanding of larval dispersal and the geographic scale of connectivity (Review by Almany et al. 2009). However, existing reserve networks have not incorporated empirical estimates of larval dispersal in their design, and may therefore fail to protect connectivity, ensure population persistence or protect biodiversity. If the demersal fish populations of an MPA are open, then the MPA can be dependent on other areas to be seeded with new recruits. Therefore, such an MPA may be vulnerable to events outside its borders, and as a result, might not fulfill the biodiversity conservation role (Leis 2003). However, the MPA may still be successful in fulfilling its fishery replenishment role if it exports propagules into the open populations of its associated fish species, thereby providing recruits to fished areas outside its borders. In contrast, if the fish populations of a MPA are largely self-recruiting (closed), then because it supplies most of its own young, the MPA should be able to fulfill its biodiversity role, but not a fishery replenishment role (it exports few propagules, thus the scale of demographic connectivity is too small) (Leis 2003). A third MPA may fulfill both roles by having a moderate degree of self recruitment, yet still be exporting large numbers of propagules to fished areas (Leis 2003).

Rather than classifying a population as fully open or fully closed, it is more appropriate to recognize that a continuum of connectivity is closer to the truth, with populations occupying differing, time- and location-dependent positions on the continuum (Leis

2002). The challenge is to identify and quantify the factors that contribute to that positioning and to quantify the spatial scale of connectivity.

2. The Pelagic Stage of Reef Fishes

The life history of a fish can be divided into five primary periods: embryo, larva, juvenile, adult and senescent. The first four of these form a cycle and only those individuals that are especially adept at survival, or just "lucky", become senescent. Each of these life history periods can be characterized by physiological processes that largely determine the changes in morphological structure, physiological capabilities, behavioural motivation and ecological role of an individual during that time of life (Fuiman 2002).

Demersal marine teleost fishes almost universally have a complex life cycle that includes a larval stage that grows and develops in the pelagic environment for several weeks to months before settling into the demersal habitat. The pelagic and benthic stages differ in almost all characteristics from morphology to size, habitat, food and behaviour (Leis 1991). In practice, the completion of metamorphosis usually defines the boundary between the larval and juvenile periods and is determined on the basis of the fish outward appearance. Many species with pelagic larvae undergo a relatively subtle change in habit or habitat as metamorphosis finishes, such as leaving the plankton to become associated with a substrate (coral, rock, or bivalve reef or vegetation). Settlement is sometimes used as a synonym for metamorphosis, even though these two terms refer to different changes, one in habit, the other a change in form (Fuiman 2002).

The development of many ecologically important features, including fins, sense organs, skeleton and external pigmentation, occurs gradually and over a large portion of the larval period. The caudal fin is often the first fin to show signs of differentiation when the urostyle, the final segment of the vertebral column, turns upward (Fuiman 2002). The

term “flexion” is frequently used to refer to this stage of development. Complete formation of fin rays, together with decreased transparency and changes in the pigmentation to the juvenile patterns, is commonly used to designate the end of the larval period and the beginning of the juvenile period (complete metamorphosis); however, complete squamation may be a more accurate endpoint (Fuiman 2002).

2.1 Larval Identification

Species identification of early life history stages is critical for ecological and taxonomic studies of the pelagic stage of fishes (Leis and McCormick 2002). There are important ecological processes, such as dispersal, which are species-specific, with related species having different behavioural patterns (Leis 1991, Leis and McCormick 2002); incorrect species identification may lead to misinterpretations of such ecological processes (Powles and Markle 1984). Given the difficulty in distinguishing larvae of related species within plankton collections, detailed descriptions are in great need.

Early work on larval development and systematics was reviewed by Ahlstrom and Moser (1981) and Moser et al. (1984). In recent years, more guides have been published with descriptions for Indic and Pacific species (Leis and Carson-Ewart 2000b, Neira et al. 1998, Moser et al. 1984, 1996), and for the western Atlantic (Fahay 1983, Richards 2005), southeastern Atlantic (Olivar and Fortuño 1991) and North Sea (Munk and Nielsen 2005). However, larval descriptions of northeastern Atlantic and Mediterranean species are more limited, and available guides are less recent, such as Fauna Flora Golfo Napoli (D’Ancona 1931-1956), and Russel (1976). More recently, Ré (1999) compiled a series of descriptions for the estuarine ichthyoplankton of the Iberian Peninsula. Nevertheless, the lack of complete descriptions of larval stages is particularly notorious as far as reef species are concerned. In most temperate nearshore studies of fish larval assemblages,

reef species as the gobies, labrids or blennids are seldom identified to the species level and most information is given to the family level (e.g. Kingsford and Choat 1989, Brogan 1994, Palomera and Olivar 1996, Tilney et al. 1996, Sabatés et al. 2003). In this respect, Gil et al. (1997, 2002), Borges et al. (2003), Faria et al. (2002, 2005, 2006) and Monteiro et al. (2008) have greatly contributed to the complete description of the larval stage of temperate reef species, including blennids (*Lipophrys pholis*, *L. trigloides*, *Parablennius pilicornis*) and gobies (*Gobius cobitis*, *G. paganellus*, *G. xanthocephalus*, *G. cruentatus*).

One way of improving our knowledge of larval descriptions is by rearing larvae under controlled conditions (Ahlstrom and Moser 1981, Leis and McCormick 2002). For reef species that spawn benthic eggs it is relatively easy to identify males guarding the eggs, transport egg batches to the laboratory, and subsequently rear the larvae through metamorphosis in order to obtain complete descriptions of their development. However, this methodology can be time consuming and is not always successful. Additionally, it must be considered that laboratory-reared larvae often differ from field-caught larvae with respect to their pigmentation or other morphological characteristics (Leis 1993), such as body proportions and meristic characters. If descriptions are based on lab-reared material, it is desirable to include information on field specimens as a means of showing similarities or differences between the two types of larvae (Leis 1993).

The use of genetic markers is proving to be a powerful tool for the correct identification of larvae collected in the plankton. For the northeastern Atlantic Ocean and the Mediterranean Sea there is a considerable number of fish families for which genetic markers are available that can be used to identify individuals, including embryos and larvae, with high levels of certainty (e.g. gobies; Monteiro et al. 2008). Coupling morphological and meristic descriptions from plankton collections with genetic validation

represents a powerful tool to increase the rate at which much-needed larval fish descriptions might be obtained in order to support ecological and evolutionary studies.

2.2 Life Strategies: Demersal *vs*. Pelagic Spawners

Reef fishes are often classified as either pelagic or demersal spawners (Thresher 1984). These reproductive modes traditionally have been viewed as distinct, alternative "strategies" to maximize larval survival and return to a population (Johannes 1978, Barlow 1981, Doherty et al. 1985), or simply as consequences of variation in adult size (Thresher 1984). Pelagic eggs are generally smaller than demersal eggs, and usually produce smaller larvae (3 to 5 mm; Thresher 1984), with less-developed sensory systems and swimming abilities (Blaxter 1986, Miller et al. 1988, Fuiman 2002). It is highly probable that the newly hatched larvae may undergo passive dispersal before becoming functionally competent. Conversely, demersal spawners incubate their eggs on the reef, providing some degree of parental protection from predators during the vulnerable embryonic period (Fuiman 2002). Larvae hatching from demersal eggs are of a larger size (5 to 10 mm; Thresher 1984), usually with functional fins, eyes and guts (Barlow 1981, Hunter 1981, Thresher 1984).

Associated with pelagic and demersal reproductive strategies, there are also potential differences in larval dispersal, duration, and patterns of settlement (Sponaugle and Cowen 1994). Several studies conducted in tropical and temperate reefs have found that larvae that are more abundant nearshore hatch from non-pelagic eggs, whereas those that are more widely distributed are generally derived from pelagic eggs. In Hawaii, Leis and Miller (1976) found that the inshore assemblage was mainly composed of reef fish larvae hatching from benthic eggs, while offshore larvae were mainly from species laying pelagic eggs. In Lizard Island region, Australia, Leis and Goldman (1987) reported a

greater abundance of larvae of reef species that spawn demersal eggs close to shore, and larvae of species which spawn pelagic eggs were more abundant in the open waters of the Great Barrier Reef lagoon. In temperate rocky reefs, studies of nearshore larval fish assemblages are scarce, but some studies support the same evidence. On the Canadian west coast, larvae from demersal eggs were dominant inshore and had a restricted alongshore distribution (Marliave 1986). In contrast, larvae originating from pelagic eggs were more uniformly distributed both alongshore and offshore. Off southwestern Nova Scotia, larvae from demersal eggs dominated the inshore shallow-water environment, while densities of larvae originating from pelagic eggs were not correlated with bathymetry (Suthers and Frank 1991).

The nearshore abundance of larvae hatched from non-pelagic eggs is thought to be due to the absence of passive drift during the egg phase (Hickford and Schiel 2003). However, in some cases, in spite of the higher abundance of larvae found close to shore, distribution patterns of larval assemblages have been shown to be weakly related to the spawning mode of adults. In northeastern New Zealand, Kingsford and Choat (1989) noted that the pelagic—demersal retention distinction was not consistent, as the distribution of several demersal-spawning families was not influenced by the proximity of reefs. Off central New South Wales, Australia, larvae from taxa with demersal eggs were more abundant close to shore (Gray 1993). However, some larvae that originated from pelagic eggs also predominated nearshore. In the Gulf of California, Mexico, several families, including sandy bottom and reef fish species, utilized the near reef habitat throughout their development (Brogan 1994). These families all spawned non-pelagic eggs and had well-developed hatchlings, but the larvae of other families, with similar spawning patterns, were not retained. Hickford and Schiel (2003) conducted ichthyoplankton surveys on the east coast of the South Island, New Zealand, and for several taxa that hatch from marine

demersal eggs, the authors rejected the hypothesis that reef fish larvae hatching from non-pelagic eggs were retained mostly or exclusively near reefs, on an exposed coast. Borges et al. (2007 a, b) found the same evidence along the rocky shore of the Arrábida Marine Park (western Portugal). These authors sampled in inshore and offshore waters and concluded that distribution patterns were independent of the spawning mode of the species.

Thus, the distribution patterns obtained in several of these studies prove to be quite variable and species specific (Cowen 2002), and therefore, it is not realistic to infer larval dispersal abilities based solely on spawning strategies. Larval retention and/or dispersion will depend on complex interactions between biological and physical factors, with some nearshore environments having particular oceanographic features that can facilitate larval retention (Harris et al. 1999, Pineda 2000, Sponaugle et al. 2002, Largier 2003).

2.3 Larval Behaviour Relevant for Dispersal/Retention

The behaviour of larval fishes has largely been ignored in considerations of dispersal. Until very recently, research focused mainly on feeding behaviour (e.g. Munk and Kiorboe 1985, Batty et al. 1990, Batty and Hoyt 1995), which does not influence dispersal outcome, and vertical behaviour. Vertical positioning of larvae in the water column will have a decisive influence on dispersal because velocity differ among depths and larvae may position themselves vertically to move horizontally in different directions or speeds (Sponaugle et al. 2002). Yet, the traditional view was that vertical position in the water column made no difference to horizontal movements of larvae. This simplifying assumption (Leis 2002) presupposed that: (1) larvae are poor swimmers that can only drift passively with currents; (2) the only biological variable of interest during dispersal is the pelagic larval-stage duration; (3) when larvae are competent to settle, they settle onto

the first bit of suitable habitat they are pushed into by the current; and (4) to the extent that larvae have behaviour of relevance to dispersal, all larvae behave the same, independently of species, ontogenetic stage or location (Leis 2006). However, several factors seem to contradict the simplifying assumption view. Distributional studies of larval fishes frequently show that larvae of different species originating from the same sort of habitat have differing distributions (Reviews in Leis 1991, Boehlert 1996, Cowen and Sponaugle 1997), which should not be the case if the simplifying assumption of passive larval behaviour applies. Several authors speculated that behaviour might be responsible (Leis and Miller 1976, Leis 1982, Richardson and Pearcy 1977, Suthers and Frank 1991), but there was little or no hard information on behaviours of the larvae, other than their vertical distribution. This scenario changed in the last two decades, as research on the pre-settlement stages of demersal reef fishes revealed remarkable behavioural abilities. In the mid 1990s, work on larvae of coral-reef fishes began to show considerable swimming abilities in perciform larvae approaching settlement, both in terms of speed and endurance. In the case of speed, this was based on observations of larvae in both the laboratory and *in situ* (Stobutzki and Bellwood 1994, 1997, Leis et al. 1996, Leis and Carson-Ewart 1997). This elicited a reassessment of the influence that behaviour of fish larvae might have on dispersal, and, in turn, an interest in the behavioural capabilities and ontogeny of behaviour in marine fish larvae. At about the same time, renewed focus began to be applied to the question of demographic connectivity in populations of marine fishes, and evidence started to emerge that self-recruitment, far from being the rare exception to demographic panmixia, was, in fact, common (Jones et al. 1999, Swearer et al. 1999, 2002). This has led to a search focused on the mechanisms that allow for self-recruitment and restricted dispersal, and, inevitably, to more interest in behaviour of pelagic larvae as a potential influence on these phenomena.

The recent findings regarding behavioural abilities, particularly those conducted on coral reef-fish larvae, have led to the replacement of the simplifying assumption by an "emerging view" (Leis 2002). According to Leis (2002) an emerging view for reef-fish larvae consists of four elements: (1) reef-fish larvae are not passive, rather they have considerable influence over their advection trajectories during the pelagic phase; (2) far-field currents are only part of what determines dispersal, and are least relevant in the early portions of the pelagic phase when larvae are most likely to be effectively passive; circulation at small scales is what influences dispersal most during the early portion of the pelagic phase; (3) at scales relevant to ecology and management, populations of coral-reef fishes are closer to the closed than the open end of the open/closed continuum, i.e., populations can present some degree of self-recruitment ; (4) long distance dispersal may not normally be very relevant to ecological and management questions. The reason for first believing that reef-fish populations were indeed open in a relevant way to management was because studies of the population genetics of coral-reef fishes commonly find genetic homogeneity over large areas (e.g. Shulman and Birmingham 1995).This evidence has been interpreted to mean that strong connectivity exists at that geographic scale, so that geographic scale is the appropriate one for management (Leis 2002). Presumably, if larval fish dispersal can maintain genetic connectivity over vast distances, then one is dealing with one population, and management at that large scale is seemingly appropriate (Leis 2002). However, amount of larval-fish connectivity required to maintain such genetic connectivity is on the order of a few individuals per generation (Shulman 1998). This is hardly enough to maintain a marine protected area (MPA) or to reseed a fishery. Therefore, the emerging view implies that for a given MPA, it may be preferable to protect more smaller parcels of reefs that large ones because ecologically-effective dispersal may not take place over large scales (Leis 2002).

In short, the major differences between the simplifying assumption and the emerging view concerns the scale of the physical-oceanographic processes emphasized, and the role and types of larval behaviour involved. The emerging view emphasizes the role of physical oceanography on scales of meters to a few kilometers and considers other behaviours, including effective swimming and larval sensory abilities related to navigation and settlement selectivity, at least as important as vertical positioning of settlement habitats (Leis 2002).

2.4 Swimming Behaviour

The swimming abilities of larval fishes are important for their survival, as they can potentially infuence their ability to avoid predators and capture prey (Reidy et al. 2000), settle to suitable juvenile habitats (Montgomery et al. 2001), control dispersal patterns (Stobutzki and Bellwood 1997), and influence levels of self-recruitment in some marine populations (Sponaugle et al. 2002). Therefore, the knowledge of swimming abilities of pelagic larval fishes assumes major importance in understanding dispersal and connectivity of reef-fish populations and is must be considered in realistic models of dispersal. The extent to which swimming by larvae influences dispersal trajectories is context-dependent, since current speeds vary spatially and temporally. Larvae are considered "effective swimmers" when they swim faster than the mean ambient current speed (Leis and Stobutzki 1999). Effective swimming is obviously easier to achieve in an area where mean current speed is 10 cm s^{-1}, than where it is 100 cm s^{-1} (e.g. Leis and McCormick 2002, Fisher 2005). However, horizontal swimming speeds lower than 'effective speeds' can strongly influence dispersal trajectories if swimming direction is normal to the current direction (Leis 2006).

Numerical models of circulation indicate that only modest speeds (1 to 10 cm s^{-1}) are required to have large effects on larval dispersal (e.g. Pepin and Helbig 1997, Wolanski et al. 1997, Porch 1998). Average current speeds on reefs range from around 5 cm s^{-1} (Pitts 1994) up to 25–30 cm s^{-1} (Wolanski and Pickard 1983). Maximum current speeds may be considerably greater (100 cm s^{-1}; Cowen and Castro 1994); however, if larvae behave similarly in the field to those simulated in modelling exercises, and show any kind of oriented swimming behaviour, they may have the potential to actively retain their position near their home reefs (Leis 2006). Additionally, larvae may also combine oriented swimming behaviour with active vertical migration, avoiding faster current flows by positioning themselves near the substratum, where currents are less strong (see Armsworth 2001), and potentially avoiding advection away from their natal reefs (Fisher and Bellwood 2003).

Swimming ability in fishes is generally classed into three types, including: 1) burst, which uses exclusively anaerobically powered muscles and lasts for less than 20 seconds; 2) prolonged, which may include both aerobic and anaerobic muscle activity lasting from 20 seconds to 200 minutes; or 3) sustained, consisting of aerobically powered muscle activity lasting for longer than 200 min (Videler 1993, Webb 1994, Kolok 1999, Plaut 2001, Fisher and Leis 2009).

Burst speeds are directly relevant to the ability of larvae to avoid predation, capture food, as well as in any other situation of sudden disturbance and maneuvering through strong current fields (Reidy et al. 2000). Therefore, burst speed is an important measure of swimming ability relevant to the ecology of larval fishes. It is the fastest speed of which an individual is capable, and is usually initiated in a C-type fast-start response (Eaton and DiDomenico 1986). Most commonly, burst speeds are measured in the laboratory as a

startle response - a fast-start behaviour that most larvae exhibit when startled by a perceived threat. A larva's startle response is the primary means by which it can influence a predator's capture success (Fuiman 1989). It can be triggered by visual or acoustic stimuli (e.g. Blaxter and Batty 1985, Batty 1989, Fuiman et al. 1999), as well as mechanical (e.g. Blaxter and Batty 1985, Blaxter and Fuiman 1989, 1990) and tactile stimuli (e.g. Blaxter and Batty 1985, Yin and Blaxter 1987).

Sustained swimming speed is performed without resulting in muscular fatigue. It includes cruising speed (e.g., migrating fishes) and speeds employed in routine activities such as spontaneous swimming, foraging and station holding (Reidy et al. 2000).

Prolonged swimming speed is very difficult to separate from sustained swimming speed in the natural habitat because rarely, if ever, can fatigue be assessed in the field (Plaut 2001). Alternatively, prolonged swimming speeds are most accurately measured in the laboratory in a swimming tunnel (Beamish 1978), because fatigue is easy to determine.

There are a great variety of methods to access swimming abilities of fish larvae. The most common measurements include routine speed, *in situ* speed, critical speed (U_{crit}) and endurance swimming speeds. This methodological diversity makes comparing values of prolonged and sustained ability obtained from different studies, leading to misleading comparisons of swimming ability among taxa or locations, or to applications of inappropriate measures of ability for a particular ecological context (Fisher and Leis 2009). In this sense, herein it will be described each type of swimming measure and its ecological relevance, instead of swimming type.

2.4.1 Spontaneous Swimming

Spontaneous swimming reflects the undisturbed day-to-day speeds and behaviour of larvae. Two different measures of spontaneous swimming are commonly used, routine speed, measured in the laboratory, and *in situ* speed, measured in the field.

The primary function of routine swimming is foraging (Blaxter and Staines 1971, O'Brien 1979, Fuiman and Webb 1988), as well as migration and predator avoidance (Blaxter and Staines 1971, Hunter 1972, Blaxter 1986). To measure routine speed in the laboratory, larvae are placed in containers, usually in still water, without natural stimuli and cues, and filmed from below or above (Fuiman et al. 1999, Plaut 2000, Fuiman and Cowan 2003, Smith and Fuiman 2004). In this type of measurement, the larva chooses the speeds exhibited, and the data usually include periods when the larva does not move; thus, these observations comprise a behavioural component other than the larva's ability to reach and maintain a particular speed. The resultant measures of speed describe the overall distances the larvae travel during their "routine" activity. It is the speed which represents the distance they would cover during searching behaviour and/or horizontal movements. Published routine speeds of larval fish are typically quite low, in the vicinity of 1 to 5 bl s^{-1} (body lengths per second) and seldom exceeding 5 cm s^{-1} (Leis 2006). Absolute routine swimming speeds (cm s^{-1}) appear to increase relatively linearly throughout larval life in relation to size, whereas relative speed, calibrated to the size of larvae (measured in bl s^{-1}), remains relatively constant for any given species.

Considerable variability exists in the development of routine swimming speed among taxonomic families. In general, it appears that routine speeds of the larvae of perciform fishes are greater than those of clupeiform, gadiform or pleuronectiform fishes, especially when expressed as bl s^{-1} (Leis 2006).

Although widely used, the laboratory procedure for measuring routine swimming speed is not standardized, which makes comparisons among studies difficult. Factors such as tank size and volume (von Westernhagen and Rosenthal 1979, Theilacker and Dorsey 1980), food availability (Munk and Kiorboe 1995, Puvanendran et al. 2002), light conditions (Batty 1987), turbulence (MacKenzie and Kiorboe 1995, Utne-Palm 2004), variability in individual behaviour, among others, might influence swimming speed of larvae and should be evaluated when comparing study results.

The second measure of spontaneous swimming behaviour is measured in the field: *in situ* speed is chosen by the larva, and is measured by SCUBA divers following larva that have been released into their natural environment (Leis et al. 1996, Leis and Carson-Ewart 1997). These observations allow new insights into factors that would be extremely difficult to recreate and investigate in the laboratory, such as the influence that different spatial factors (e.g., near or away from settlement sites), environmental factors (e.g., time of day or sensory cues) or swimming directions can have on swimming speed. *In situ* speed is difficult and labor-intensive to measure, but it is the only method to follow and measure swimming speed of fish larvae in the ocean. The released and observed larvae may be either laboratory-reared (Leis et al. 2006a, b, 2009 a, b), or wild individuals that are previously captured with light traps or nets (Leis and Carson-Ewart 1997, 2000a, Trnski 2002, Leis and Fisher 2006). The behaviour of larvae may be influenced by the presence of the divers, and at present, there is no direct way of quantifying this possible influence. However, a number of observations provide circumstantial evidence that *in situ* speed is a reasonable measure of how larvae actually behave in the sea. *In situ* speeds are considerably less than maximum speed measurements such as U_{crit} measured for the same species in laboratory settings (Fisher and Wilson 2004, Clark et al. 2005, Leis and Fisher

2006), demonstrating that larvae being observed by divers are not trying to escape and are probably swimming at speeds that can be sustained aerobically. Moreover, larvae swim at different speeds in different situations (locations or directions) while under observation by divers (Leis and Carson-Ewart 2001, 2002, 2003, Leis et al. 2006a, b), which suggests that the observer's presence does not completely override the behaviour of larvae. Larvae also feed while being observed (Leis and Carson-Ewart 1998, Trnski 2002, Hindell et al. 2003) and take shelter when predators are present (Leis and Carson-Ewart 1998), suggesting that larvae do not respond to divers the same way they respond to predators.

Most available measurements of *in situ* speed are of settlement-stage wild larvae, mostly of coral-reef fishes (Leis and Carson-Ewart 1997, 2001, 2003, Leis and Fisher 2006), but some data on warm-temperate species are also available (Trnski 2002, Hindell et al. 2003, Leis et al. 2006b). The speeds of these settlement-stage larvae are high relative to ocean currents and vary on a taxonomic and spatial basis. Average speeds of late-stage larval coral-reef fishes measured on the Great Barrier Reef and French Polynesia were 2-66 cm s^{-1} (2-34 bl s^{-1}), depending on the species, with one holocentrid swimming as fast as 66 cm s^{-1}, and several species with mean speeds of 40 cm s^{-1} (Leis and Fisher 2006). Furthermore, *in situ* swimming speeds of 3-10 bl s^{-1} were found in reared larvae of three warm-temperate species (Leis et al. 2006b).

Measuring larval swimming speed *in situ* has some disadvantages: observations can be conducted over only relatively limited time periods (usually 10 min), only during the day, only in the upper portions of the water column, and not all species or developmental stages are amenable to this methodology.

2.4.2 Forced Swimming

Two of the most common measures of aerobic swimming ability of fishes include critical speed (Brett 1964) and endurance speed (Beamish 1978). Methods to investigate both measures consist of forcing larvae to swim against current in a swimming tunnel.

The U_{crit} measures maximum swimming speed (modified from Brett 1964). It is an easy measure of swimming performance and involves swimming fish at incrementally-increasing speeds until exhaustion (Plaut 2001). The result is a measure of maximum aerobic swimming speed maintainable over short periods. The technique has been used extensively over the last 40 years to examine the swimming speeds of fishes in relation to a variety of ecological, biological, and environmental factors (Hartwell and Otto 1991, Myrick and Cech 2000, Green and Fisher 2004), and has also been used more recently to estimate the potential effects of swimming behaviour on dispersal patterns of coral (Stobutzki and Bellwood 1994, Fisher et al. 2000, 2005, Fisher 2005, Hogan et al. 2007, Leis et al. 2009a), and subtropical and temperate reef fishes (Clark et al. 2005, Leis et al. 2006b). Swimming abilities on the scale measured by U_{crit} may be important in terms of the potential for larvae to move between locations on a small scale, such as away from reefs at hatching, within slicks or between plankton patches in the open ocean, or between habitats at settlement (Fisher et al. 2000). Although many species have U_{crit} values in excess of average ambient current speeds (Fisher 2005, Fisher et al. 2005, Hogan et al. 2007), it is unlikely that these speeds could be maintained for the length of time required to substantially affect overall dispersal patterns. Therefore, U_{crit} is not directly applicable to field situations, but is a useful measure of *relative* speed, for comparisons among taxa or developmental stages. U_{crit} values are often correlated with other measures of swimming performance that are critical to larval survival, including routine swimming activity (Plaut 2000, Fisher and Bellwood 2003) and sprint swimming speeds (Reidy et al.

2000). Furthermore, U_{crit} swimming ability is closely correlated with the swimming speeds that larvae are able to maintain for many hours (Fisher and Bellwood 2002, Fisher and Wilson 2004) as well as *in situ* swimming speeds (Leis and Fisher 2006). Consequently, U_{crit}, while simply a measure of short-term maximum speed, may also provide insights into other ecologically-important abilities of larvae.

Most research on fish larval critical swimming ability has focused on coral-reef species (e.g. Stobutzki and Bellwood 1994, 1997, Fisher et al. 2000, 2005, Fisher 2005, Leis et al. 2007, 2009a, b) and, to a lesser extent, temperate fishes (e.g. Dudley et al. 2000, Clark et al. 2005, Leis et al. 2006b, Faria et al. 2009, Koumoundouros et al. 2009, Pattrick and Strydom 2009). U_{crit} of settlement-stage coral-reef fish can range from 5 to 100 cm s^{-1} (Fisher et al. 2005). Temperate fish larvae have comparatively lower critical speeds (e.g. 1-25 cm s^{-1}, Clark et al. 2005, 2-35 cm s^{-1} Pattrick and Strydom 2009), but still, they are considerably better swimmers than previously assumed (e.g. Blaxter 1986).

Endurance swimming, as a measure, does not fit into any of the three categories described earlier – burst, prolonged or sustained (Videler 1993). However, much of the initial work on swimming abilities in late-stage larval coral-reef fishes was based on an endurance swimming technique (Stobutzki 1997, 1998, Stobutzki and Bellwood 1997) and these studies have proved that late-stage larval fishes are capable of sustaining relatively fast swimming speeds (over 10 bl s^{-1} for some species) for long periods of time (days) and covering very long distances (100's of kilometers in some cases). Like U_{crit}, this is a measure of potential performance of larvae, but over considerably longer periods. It measures the time over which a fish can swim constantly against a given water velocity. Settlement-stage larvae of coral-reef fishes are capable of swimming for days and for 10s of km at a fixed speed. The currently accepted standard-reference speed of 13.5 cm s^{-1}

was first chosen by Stobutzki and Bellwood (1997) to test endurance duration as this was the mean current speed in the vicinity of Lizard Island, on the Great Barrier Reef, where their work took place. Swimming abilities of wild, settlement-stage larvae of over 20 species from nine families (Acanthuridae, Apogonidae, Chaetodontidae, Lethrinidae, Lutjanidae, Monacanthidae, Nemipteridae, Pomacanthidae, Pomacentridae), were tested in the raceway, resulting in average mean family values of 84h of swimming (range: 7.4-194 h) and 41Km distance (range: 3.6-94 km).

The results for settlement-stage coral-reef fish larvae were similar to those obtained for species from temperate reef systems, which also showed remarkable swimming endurance. Swimming times and associated distances varied among the species examined, but ranged from as high as 200 km (Scorpidae) down to 50 km (Monocanthidae; Dudley et al. 2000). These initial studies have changed the traditional belief that these larvae were poor swimmers, and raised awareness of the potential effect of larval behaviour on dispersal patterns, particularly in the later stages of development (Wolanski et al. 1997, Armsworth et al. 2001). However, despite the importance of this sustained-swimming data in increasing our understanding of larval capabilities, such data are still insufficient to be included into dispersal models, because endurance swimming times and distances vary substantially with swimming speed (Fisher and Bellwood 2002, Fisher and Wilson 2004), and also because swimming times and distances can be greatly increased in the presence of food (Fisher and Bellwood 2001, Leis and Clark 2005). It is unlikely that larvae swim continuously in the field without feeding or resting, so one can speculate that in the wild, larvae will have substantially greater endurance values. An increase of 1.8-2.4 fold in endurance was found in a single pomacentrid species (*Amphiprion melanopus*) when fed (Fisher and Bellwood 2001), with some individuals not reaching exhaustion, and growing as fast as undisturbed larvae. In six other pomacentrid species, the

experiment was terminated after a 2- to 5-fold increase in endurance over unfed larvae was attained with the larvae still swimming strongly (Leis and Clark 2005), indicating that endurance is less limited by fatigue than by energy supplies.

A modification of critical swimming speed and endurance speed is called maximum sustainable speed (Fisher and Wilson 2004) and is measured by swimming larvae at a single speed for a period of 24 hours. Although this is not a widely-used technique for measuring swimming performance, this measure takes into account that larvae will have the greatest impact on their dispersal if they swim at the fastest speed they can sustain without exhibiting the effects of exhaustion. The 24-hours period was first selected because starvation is not expected to be an issue, yet the duration is long enough to show convincingly that the experimental speed is sustainable over time periods long enough to be relevant to dispersal. Studies on sustainable speed are important in defining the true "maximum" potential of these larvae to influence dispersal patterns in the open ocean using swimming behaviour. Estimates of maximum sustainable speed are available for the settlement-stage larvae of nine species of three families (Apogonidae, Lethrinidae and Pomacentridae) of tropical reef fishes, and range from 8-24 cm s^{-1} (5-14 bl s^{-1}; Fisher and Wilson 2004). These are about one-half of U_{crit} measurements of the same species (Fisher and Wilson 2004) and similar to values of *in situ* speed reported for settlement-stage larvae of the same or related species (Leis and Fisher 2006). Data on maximum sustainable speeds are lacking for temperate species.

2.4.3 The Most Relevant Speed for Dispersal

As described in the previous sections, there is a great variety of swimming measurement techniques which provide useful information in terms of spatial location behaviour of larval fishes. Yet, the question remains: which is the speed most relevant for

considerations of dispersal? Routine speed has the advantage of being a measure of swimming speed undisturbed by divers or any evident forcing by the investigator, but it carries the disadvantage of being measured in artificial laboratory conditions. U_{crit} is most relevant for comparisons of relative performance, but it is not a performance measure that can be directly included in dispersal models, and it almost certainly overestimate what larvae actually swim in the sea. Endurance experiments measure long-term swimming performance and provide data directly relevant to larval fish dispersal. However, direct application of these endurance performances to the field is not possible because it is unlikely that larvae *in situ* swim to exhaustion without food or rest, and when larvae can feed, endurance is essentially open-ended (Fisher and Bellwood 2001, Leis and Clark 2005). *In situ* speed has the clear advantage of being measured in the sea, but with the unknown influence of the observing divers. In spite of this, *in situ* speed is the probable the most realistic measure of how fast larvae actually swim in the sea, and is, therefore, the most relevant for dispersal models.

Considering the limitations of each technique and considering that applying the four described methodologies in a single study is not logistically feasible in most circumstances, it is important to discuss how these measures relate to each another. A comparison of swimming speeds of fish larvae made by different methods and on different species will allow several conclusions and calibration. Average routine swimming speeds appear to be around 20% of U_{crit} (Fisher and Bellwood 2003) and provide an important estimate of the minimum undisturbed speeds at which larvae swim on a daily basis. Such values may be useful for considering the minimum impacts that larval behaviour may have on dispersal, but it is likely that larvae will swim in the ocean at higher speeds (Fisher and Leis 2009). More work is needed to examine the generality of these relationships over a wider range of species and under varying laboratory

conditions. *In situ* speeds are also usually greater than routine speeds, but lower than U_{crit} speeds. This measurement provides important insight into how the swimming speeds of larvae vary in the open ocean in response to a range of environmental sensory cues (Leis and Carson-Ewart 1998). There is a strong positive relationship between *in situ* speed and U_{crit} (Leis and Fisher 2006), and so U_{crit} may be used to predict *in situ* speed from laboratory measurements of potential speed for stages or taxa that are not amenable to *in situ* methodology. The maximum *in situ* swimming speeds of larvae appear to be around 40-50% of their U_{crit} speeds (Leis and Fisher 2006) and they range substantially among species as well as among individuals within species. Endurance studies on coral-reef fish larvae show that maximum sustainable swimming speeds lie in the region of 50% of the U_{crit} speeds of late-stage larvae for a variety of species (Fisher and Bellwood 2002, Fisher and Wilson 2004); this relationship between endurance and U_{crit} appears to be consistent during ontogeny for the few species for which data are available (Fisher and Bellwood 2002).

2.4.4 Ontogeny of Swimming Behaviour

How and when behaviours that are important to the dispersal or retention of larvae, such as swimming and orientation, develop during the pelagic larval period is largely unknown. Most work to date on swimming capabilities of the larvae of demersal fish species has concentrated on larvae nearing settlement (Stobutzki and Bellwood 1994, 1994, Leis and McCormick 2002, Jenkins and Welsford 2002, Trnski 2002, Leis and Carson-Ewart 2003). Although this provides valuable information on the final portion of the pelagic larval phase, smaller, younger, less developed larvae are unlikely to have equivalent performances. This dearth of knowledge about the ontogeny of behaviour in larval fishes inhibits the development of realistic dispersal models. Current work is thus

now directed at understanding the ontogeny of behaviour (e.g. Clark et al. 2005, Leis et al. 2006 a, b, 2009 a, b, Faria et al. 2009); the general trend indicated by such studies is for swimming speed to increase positively with larval size, in absolute terms. In interspecific comparisons, size is a much better indicator of developmental stage than age (Fuiman et al. 1998), and so it is useful to examine developmental rates of swimming ability with respect to larvae length, rather than time since hatching. However, caution should be taken when making inter-specific comparisons because developmental time decreases with increasing temperatures in many fish species (Blaxter 1969) and the same ontogenetic events may take place in larvae of different sizes at different temperatures. Since developmental time decreases with increasing temperatures in many fish species (Blaxter, 1969), the same ontogenetic events may take place in larvae of different sizes at different temperatures. The ontogenetic index, proposed by Fuiman (1994) is a good tool to allow inter-specific comparisons on the basis of larval size or age at a certain ontogenetic event (Fuiman 1994, Fuiman et al. 1998).

Studying behavioural ontogeny poses a new challenge, as it requires sampling larvae representing a wide variety of ontogenetic stages (sizes). Late-stage larvae of some species are easily captured in good condition using light traps (Doherty 1987) or fixed nets (Dufour and Galzin 1993). On the other hand, younger larvae can only be captured usually using plankton nets and are more susceptible to injury or death in the process (Leis et al. 2006b). For that reason, studies of younger larvae have focused on species that can be reared in captivity (Leis et al. 2006b). An obvious and important question is whether the behaviour, or specifically swimming performance, of reared larvae is similar to that of wild larvae. From the available data, the answers are mixed. It is generally agreed that behaviours with a learned component, such as anti-predator behaviour, will differ between wild and reared individuals because larvae from the two sources have

experienced very different conditions (Olla et al. 1998, Brown and Laland 2001). However, some studies that investigated swimming behaviour present contradictory results. For example, reared fish may swim faster or slower, or they may perform equivalently to wild fish, with ontogenetic variations further complicating this picture (von Westernhagen and Rosenthal 1979, Danilowicz 1996, Smith and Fuiman 2004). So, when using results from reared individuals, it is desirable to compare the behaviour of these larvae to that of wild fish, especially if the results will be used to make inferences about fish in nature (Leis 2006).

2.4.5 Temperate *vs*. Tropical Fish Larvae

Most research on fish larval swimming abilities has focused on tropical coral-reef species and, to a lesser extent, temperate fishes. Reef fish larvae generally appear to be much better swimmers than non-reef fish larvae when short-term and prolonged activity are compared. Why are reef fish larvae thought to be better swimmers? There is undoubtedly a taxonomic component. Most studies on temperate larvae focused on gadiform or clupeiform species (Blaxter 1986, Miller et al. 1988), as opposed to tropical studies, which have mainly focused on perciform species (e.g. Fisher et al. 2000, Fisher et al. 2005, Leis and Fisher 2006), which are apparently superior performers relative to gadiform or clupeiform larvae. However, the few existent studies on swimming abilities of temperate perciform larvae (e.g. Clark et al. 2005, Faria et al. 2009, Pattrick et al. 2009) suggest performances comparable to tropical larvae.

Additionally, comparisons of swimming performances are frequently made regardless of developmental stage. The performance of pre-settlement stage larvae is not comparable to performance of younger stages. Similarly, coral-reef fish tend to be more developed at any given size than most temperate fish (Leis and McCormick 2002). This is particularly

apparent if one considers the state of development of well-studied temperate larvae such as herring and cod at the sizes at which reef fish larvae commonly settle (1-2 cm). At all sizes, the reef-fish larvae have more complete fins. They develop scales at a smaller size, seemingly having better developed sensory apparatus at any size, and are morphologically equipped for effective feeding within a few days of hatching and at smaller sizes than herring and cod (Leis and McCormick 2002).

Other than taxonomy and differences in developmental rate, temperature is probably the most relevant confounding factor for many of the comparisons among tropical and temperate species. Temperature can affect swimming performance of fish larvae in two ways. On one hand, fish muscle cells operate more efficiently at higher temperatures (Hunt von Herbing 2002); on the other, temperature is necessarily linked to viscosity effects that impinge upon fish larval motion (Podolsky 1994, Fuiman and Batty 1997, Hunt von Herbing 2002). Whether larvae swim in a viscous or an inertial environment has a major effect on their swimming performance because the interaction between larvae and water in viscous environments makes swimming energetically more expensive (Hunt von Herbing 2002). Given this, swimming may be more efficient in tropical waters (25-29ºC) than in temperate waters (10-20ºC).

In summary, comparisons of swimming speed among taxa should take into account phylogeny, methodology, developmental state and temperature. Most recent studies have begun to take these factors into consideration when making interspecific comparisons. As a result, the extent to which tropical larvae might swim better than temperate larvae is now unclear. An increasing number of examples have arisen to show that swimming abilities of temperate species are indeed comparable to tropical larval abilities (e.g. Dudley et al. 2000, Clark et al. 2005, Faria et al. 2009, Pattrick et al. 2009), thus reinforcing the need of taking into account taxonomic differences.

2.5 The End of the Pelagic Stage

Near the end of the larval phase fish reach a developmental stage in which they are ready to leave the water column and join the demersal reef population. This developmental stage is known as "competence" (drawing from invertebrate terminology; Cowen 1991). Thus, reef fish have a larval phase consisting of two parts: (1) a pre-competent phase, during which rapid development and growth occurs, and (2) a competent phase, characterized by reduced growth and body maintenance (Leis and McCormick 2002). Some widely-distributed families can apparently extend the period of competence, by delaying metamorphosis and the timing of settlement (Victor 1986, Cowen 1991, Jenkins and May 1994, Cowen and Sponaugle 1997, Sponaugle and Cowen 1994, 1997). Flexibility in the length of the pelagic period or the competency period might be expected to result in some benefit, perhaps ultimately better success in finding suitable settlement habitat (Leis 2006). Fish larvae are clearly selective about where they settle (Marliave 1977, Booth and Wellington 1998, Montgomery et al. 2001). Selectivity is often based on the presence of specific benthic substrata or the presence of conspecifics or other species (e.g. McCormick and Makey 1997, Ohman et al. 1998, Leis and Carson-Ewart 1999, 2000a, Holbrook et al. 2002). In this sense, habitat selection may also play a determining role in explaining spatial and temporal variation in larval fish recruitment, in addition to larval supply (Carr 1991). One of the great puzzles of coral-reef fish ecology is how the young, pelagic stages locate the relatively rare patches of coral-reef habitat on which they settle and ultimately reside as adults. The answer must lie in the sensory world of these fishes, because it seems unlikely that successful settlement is solely a matter of chance (Myrberg and Fuiman 2002). Pre-settlement stages of reef fishes have excellent locomotor capabilities that may even exceed those of recently settled individuals (Stobutzki and Bellwood 1994, Leis and Carson-Ewart 1997, Stobutzki 1998).

Furthermore, their swimming direction in the sea appears to be oriented (Leis et al. 1996, Leis and Carson-Ewart 1999, 2001, 2003, Leis et al. 2006 a, b), although the specific cue or cues to which they orient remain unknown (Leis et al. 1996, Stobutzki and Bellwood 1998, Leis and Carson-Ewart 1999). Recent studies have suggested a number of cues that larval fishes may use to detect a suitable habitat from distant locations. A primary consideration is the large distance over which the signal must be detected, on the order of kilometers or perhaps tens of kilometers, which essentially eliminates lateral line and visual cues. Vision can operate over only limited spatial scales (tens of metres) for orientation in the ocean due to the attenuation of light. Over these scales, it is most likely to be useful for vertical distribution and settlement behaviour; both have been demonstrated (Myrberg and Fuiman 2002, Kingsford et al. 2002). The lateral line system and its developmental precursors may be able to mediate rheotaxis, but orientation to currents cannot operate without an external reference (Montgomery et al. 1997). Evidence to date supports only two potential cues that might operate over kilometer-scales: chemicals (Atema et al. 2002, Wright et al. 2005, Dixson et al. 2008) and sounds (Simpson et al. 2004, 2005, 2008, Tolimieri et al. 2004, Wright et al. 2005, Montgomery et al. 2006). Thus far, all demonstrations of sound being useful as an orientation cue have been at scales of <1 km. Hearing has the potential to operate over large distances (tens to hundreds of kilometres) as sound travels well in water with little attenuation (Popper and Carlson 1998). Further, hearing is what has been called a current-independent cue because the stimulus is not distributed by currents (Armsworth 2000). This increases the utility of sound as an orientation cue not only because it spreads in all directions, but also because weaker swimmers can utilize such a cue without necessarily needing to swim directly upstream to localize its source. The sense of smell can operate over moderate scales, but because odours are diluted (and their components perhaps degraded) with

distance from the source, and because odour is a current-dependent cue, it is potentially less useful for orientation than sound. There is no question that settlement-stage fish larvae can detect odours, can change behaviour in response to them, and can localize them, at least over scales of tens of metres (Montgomery et al. 2001, Kingsford et al. 2002, Myrberg and Fuiman 2002, Leis and McCormick 2002). As with hearing, what remains to be determined about olfaction is which substances can be detected, at in what stage in development this ability is present and over what spatial scales such orientation can actually operate.

The use of other senses, such as magnetodetection and electrodetection, for orientation by larvae of marine fishes is largely speculative, although both are known to be used by adults of some species (Myrberg and Fuiman 2002, Kingsford et al. 2002). Additionally, it is reasonably clear that gravity and pressure are used in vertical distribution behaviour, can be used over large scales, and operate at a very early stage in development.

In summary, although much remains to be learned, it is clear that several classes of sensory cues may be used by fish larvae for orientation to a reef and that the role of different senses will change as a fish draws nearer to its destination (Myrberg and Fuiman 2002).

2.6 What Influences Larval Survival?

Population dynamics of marine fishes are characterized by high larval mortality (Werner and Fuiman 2002), which is related to size- and growth-selective processes (Meekan and Fortier 1996). Size-at-hatching and growth rate strongly influence survival before and after settlement (Meekan and Fortier 1996), with higher survival of juveniles resulting from larger larvae (Houde 1994, Bergenius et al. 2002, Shima and Findlay 2002, Raventós and Macpherson 2005). Moreover, predation and starvation are major mortality

factors affecting the larval stage (Houde 1987), and changes in morphological traits can strongly influence larval survival. Search for prey and avoidance of predation are strongly related to swimming capabilities, which, in turn, depend on the size and condition of larvae (Houde 1987, Blaxter 1988, Leis and Carson-Ewart 2001). Growth during the larval period is influenced by both exogenous and endogenous factors (Jones 2002). Exogenous factors include food (e.g. Jones 1986), temperature (e.g. Blaxter 1992,), oxygen (e.g. Person-Le Ruyet et al. 2002) and salinity (e.g. Boeuf and Payan 2001). Endogenous factors include genetics and also parental influences (Green and McCormick 2005). Parental effects have been found to influence size at hatching (Kerrigan 1997, Green and McCormick 2005, Trippel et al. 2005, McCormick 2006), hatching success (Laine and Rajasilta 1999, Saillant et al. 2001, Rideout et al. 2004, Trippel et al. 2005), yolk reserves (Kerrigan 1997, Gagliano and McCormick 2007), swimming ability (Green and McCormick 2005) and growth rate of larvae (Green and McCormick 2005). In particular, maternal influences due to nutritional provisioning of the embryo (Bernardo 1996) are assumed to predominate over paternal contribution, as sperm contain virtually no extra-nuclear material. From fertilization of the egg to the onset of planktonic feeding, larvae are dependent on the energy reserves within the yolk, which in turn are a direct result of the reserves the female is able to mobilize during oogenesis and maturation of oocytes (Kerrigan 1997). The social condition under which breeding occurs has also effects on the size of the larvae through a stress-related mechanism (McCormick 2006). Pairs breeding in isolation produced larger larvae, as opposed to pairs breeding in the presence of additional females. Larger offspring may possess characteristics such as enhanced swimming ability (Miller et al. 1988, Fisher et al. 2000), an earlier time of first feeding or greater predator avoidance (Miller et al. 1988). If parental effects influence the

growth or development rate of larvae (Donelson et al. 2008), and thus size at a given age, then these influences are likely to be of major importance to offspring survival.

On the other hand, environmental variation during the early life stages can also influence larval condition (Green and McCormick 1999), performance (Green and Fisher 2004), growth and development (Sponaugle et al. 2006), and ensuing recruitment magnitude (Bergenius et al. 2005). Environmental factors encompass the physical and biotic processes acting on developing eggs and larvae, including the physicochemical conditions of the water and food availability, among others (Chambers 1997).

Much evidence indicates that the two main mortality agents acting upon marine fish larvae are predation and starvation (Bailey and Houde 1989). These factors are not necessarily independent, as starvation leads to decreased growth rate (Ehrlich et al. 1976, Yin and Blaxter 1986), slower development (Kamler et al. 1990, Høie et al. 2000), and changes in behaviour (Blaxter and Ehrlich 1974, Munk 1995, Ross et al. 1996, Sogard and Olla 1996, Chick and Van den Avyle 2000). Larvae with low nutritional status will consequently be smaller, weaker and less developed with regard to sensory and locomotory capacities than well-fed larvae of the same age, thus being more susceptible to predation. Indeed, studies indicate that larger size or faster growth of larvae can lead to enhanced recruitment or juvenile survival (Searcy and Sponaugle 2001, Bergenius et al. 2002, Shima and Findlay 2002, Vigliola and Meekan 2002, McCormick and Hoey 2004, Sponaugle et al. 2006). However, size- and growth-selective mortality may not be the only processes affecting survivorship of newly settled reef fishes. Growing evidence suggests that condition may play a role in survival (Booth and Hixon 1999, Booth and Beretta 2004, Searcy and Sponaugle 2001, Hoey and McCormick 2004). Variable condition can lead to differences in behaviour, such as swimming and foraging (Stobutzki 1997, Green and McCormick 1999, Sogard and Olla 2002). Enhanced swimming

capability associated with higher condition can result in increased response or quicker evasion during predator attacks (Chick and Van den Avyle 2000, Grorud-Colvert and Sponaugle 2006). To obtain sufficient food, recruits with lower condition may take greater behavioural risks, increasing their vulnerability to predation (Sogard 1997, Pressier et al. 2005). Recruits with higher condition may be able to survive for longer periods of time by using their greater energy reserves to mediate the effects of lost foraging time due to the threat of predation (Lima and Dill 1990). Condition, or physiological state, of a fish may be measured in a variety of ways, such as lipid content, protein, carbohydrate, standardized weight, or cell vacuolation, RNA/DNA, robustness, or by a variety of behavioural or developmental features (see McCormick and Molony 1993, Review by Ferron and Leggett 1994, McCormick 1998, Suthers 1998).

Vulnerability to predators is believed to decrease with growth rate because the number of potential predators declines and escapement abilities improve as larvae grow (Miller et al. 1988, Bailey and Houde 1989). In addition, the risk of starvation also diminishes through the course of development, not only because of lower weight-specific metabolism and increasing energy reserves, but also because of improvements in sensory and swimming performances (Fuiman 2002). Older larvae are better equipped to locate more distant food supplies. Growth also allows larvae to select larger, more energy-rich prey while retaining the ability to feed on smaller, more abundant prey.

3. Goals

The major goal of this study is to provide an important step forward in understanding how behaviour potentially influences dispersal of temperate reef fishes. It seeks to present baseline behavioural knowledge needed for future incorporation of larval behaviour into predictive dispersal models. The specific objectives addressed in this study were to:

1. Study early development of temperate-reef species (**Chapter II**);

2. Understand how critical, routine and endurance swimming ability vary among species and when these abilities develop during the pelagic larval stage (**Chapters III, IV**);

3. Investigate whether laboratory-reared larvae differ in behavioural performances from wild-caught larvae (**Chapter III-B**);

4. Evaluate the influence of nutritional condition on swimming performance of larvae (**Chapter IV**).

Chapter II represents a small contribution to the problem of larval fish identification. The correct identification of eggs and larvae is a requirement for ecological and taxonomic studies of the pelagic stage of fishes, and this chapter addresses problems related to misidentification of two reef species belonging to the Gobiesocidae.

Chapters III-A and IV-A provide insight into the ontogeny of behavioural abilities of temperate reef fish larvae, in particular swimming abilities, and how these might help explain dispersal/retention patterns of these species. Experiments were performed using laboratory-reared larvae, either hatched from eggs spawned at the laboratory or from batches of eggs brought in from the wild. An opportunity was taken to collaborate with a worldwide specialist in larval behaviour (Professor Lee Fuiman, University of Texas), resulting in a study of a warm-temperate reef fish that was accomplished with the objective of comparing swimming performance of wild and laboratory reared larvae (**Chapter III-B**). To access the influence of nutritional condition on swimming behaviour, dispersal potential and ultimately on recruitment, a study was conducted using two commercially-important species (**Chapters IV-A, B**).

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CHAPTER II. EARLY DEVELOPMENT OF TEMPERATE REEF FISHES

Early development and larval behaviour of two clingfishes, *Lepadogaster purpurea* and *Lepadogaster lepadogaster* (Pisces: Gobiesocidae)

Tojeira, I.; Faria, A.; Henriques, S.; Faria, C.; Gonçalves, E. Early development and larval behaviour of two clingfishes, *Lepadogaster purpurea* and *Lepadogaster lepadogaster* (Pisces: Gobiesocidae). *To be submitted*

ABSTRACT

The recent revision on the taxonomic status of *Lepadogaster lepadogaster* led to a clarification of the subspecies status of *L. l. lepadogaster* and *L. l. purpurea*, proposing *L. lepadogaster* and *L. purpurea* as valid species and the elimination of *L. zebrine* with its synonymy to *L. lepadogaster*. This new taxonomic status led to the need of clarifying the early development of the two species, since the few available descriptions did not allow to confidently ascribing the observed larvae to each of these species. We therefore raise broods of the two species in captivity and described the detailed embryonic, larval and behavioural development. Embryonic development lasted 21 days in *L. purpurea* at a mean temperature of 14.2º C, and 16 days in *L. lepadogaster*, at a mean temperature of 16.5º C. Newly hatched larvae of both species measured 5.2 mm, had the mouth and anus opened, pigmented eyes and almost no yolk. The two species can be differentiated at hatching and throughout development by the pigmentation on the ventral region, which is absent in *L. purpurea*. The change to a benthic mode of life was gradual in both species, with larvae increasingly spending more time close to the bottom until definitely settling. Larval development lasted 33 days in *L. purpurea*, at a mean temperature of 14.6º C, and 18 days in *L. lepadogaster*, at a mean temperature of 16.5º C. In addition, some notes on larval behaviour are provided.

KEY-WORDS: *Lepadogaster lepadogaster*, *Lepadogaster purpurea*, gobiesocidae, clingfishes, early development, larval behaviour

INTRODUCTION

The Gobiesocidae or clingfishes have a worldwide distribution, occurring in many different habitats in tropical and temperate seas (Briggs, 1955, 1986, 1990). However, knowledge of their behavior (Gonçalves *et al.*, 1996, 1998) and ecology (Henriques *et al.*, 2002) is extremely poor. This is related to their small size, which enables them to occupy very cryptic microhabitats (Thresher, 1984). These species possess a ventral sucking disk which provides extra adaptation to explore crevices, holes and narrow spaces between rocks, as well as resist to strong water movements (which are prevalent in intertidal and shallow subtidal habitats). Demersal eggs are deposited on the underside of stones, with the male guarding the egg mass which may contain multiple batches at different stages of development (Breining & Britz, 2000).

Lepadogaster purpurea (Bonnaterre, 1788) and *L. lepadogaster* (Bonnaterre, 1788) are two abundant species of clingfishes (Briggs, 1955, 1986). Known distributional patterns range from Scotland to Senegal, the Canary Islands and Madeira Islands and the Mediterranean for *L. purpurea* and at least as far north as the extreme north-west of Galiza to north-west Africa, also occurring in the Canary Islands and Madeira Islands and in the Mediterranean for *L. lepadogaster* (Henriques *et al.*, 2002). These are very closely-related species quite similar in morphology. In adults, they can be distinguished by the different head marks (or *ocelli*), as well as the number of the papillae of the sucking disc regions. The different body colouration patterns, the length of the nostrils and the distance between eyes are other distinctive characters used to identify each species (Henriques *et al.*, 2002). They differ in microhabitat preferences with both species occurring in rocky boulder fields of the intertidal and subtidal zones down to 7m depth (but *L. purpurea* sheltering in larger boulders and somewhat deeper than *L. lepadogaster*) (Henriques *et*

al., 2002). The most striking difference between these species is however the breeding period. *L. purpurea* breeds mainly during the winter until the beginning of the spring (October to April) and *L. lepadogaster* breeds mainly during the spring until the beginning of the summer (March to July) (Henriques *et al.*, 2002).

Due to this close resemblance, *Lepadogaster lepadogaster* was considered until recently one single species, with two subspecies: *L. lepadogaster lepadogaster* and *L. lepadogaster purpurea (*Henriques *et al.*, 2002). These authors revised the taxonomic status of *L. lepadogaster* and divided this species into two different ones: *L. lepadogaster* and *L. purpurea*. This taxonomic confusion and geographic overlap renders the previously scattered descriptions of the early stages of *Lepadogaster* (Guitel 1888) useless and clarification is needed in order to correctly ascribe the right larvae to the right species.

The objective of this study is therefore to clarify the early development of the two species of *Lepadogaster* (*L. lepadogaster* and *L. purpurea*) providing a detailed description of the embryonic and larval stages, which is a central requirement for ecological and taxonomic studies of the pelagic stage of fishes (Leis & McCormick, 2002).

MATERIALS AND METHODS

Fourteen specimens of *L. purpurea* were captured during the months of January and February 2006, and fourteen specimens of *L. lepadogaster* were captured in April 2006, during the breeding season of each species, at Alpertuche beach (38º28’ N; 8º59’ W), located at the Arrábida Marine Park (Portugal). Individuals were kept in a 250 l tank illuminated with fluorescent light (60 W) 12 h per day, and were fed twice a day with a varied diet. Water temperature was kept at 13º C for *L. purpurea* and 15º C for *L.*

lepadogaster, according to the sea temperature at the sampling site. The substratum included several layers of sand, with small (5-10 cm) and large (20-30 cm) stones. Shelter was formed by flat rocks that were also used as breeding sites by the males.

For each species, 8 batches were obtained. The complete embryonic development sequence for *L. purpurea* was based on one batch laid on 17 March 2006 (mean water temperature ± S.D. = 14.2 ± 0.67º C, range = 13-15º C, n = 20), and for *L. lepadogaster* on two batches laid on 1 June 2006 (mean water temperature ± S.D. = 16.5 ±0.46º C, range = 16-17º C, n = 16) and 11 June 2006 (mean water temperature ± S.D. = 16.5 ± 0.43º C, range = 16-17º C, n = 16). The remaining batches were used to complete or confirm specific developmental features and were not sampled on a daily basis. No significant deviations from the patterns observed on the main batches used for descriptions were observed. Eggs were collected daily for description: the egg capsules were opened and the embryos distended to allow more detailed observations. Total egg mean number by batch, egg density and egg mass area was calculated for each species.

Larval development sequence was described based on two batches for each species. The batches of *L. purpurea* hatched on 6 February 2006 (mean water temperature ± S.D. = 14.6 ± 0.54º C, range = 13-15º C, n = 39) and 3 March 2006 (mean water temperature ± S.D. = 14.8 ± 1.68º C, range = 13-23º C, n = 32), and batches of *L. lepadogaster* hatched on 1 June 2006 (mean water temperature ± S.D. = 17.1 ± 0.43º C, range = 16-18º C, n = 33) and on 13 May 2006 (mean water temperature ± S.D. = 17.8 ± 0.32º C, range = 17-18º C, n = 34). Upon hatching, larvae were collected by aspiration from the parental aquarium and reared in 25 l tanks, illuminated with fluorescent light (15 W) 24 h per day. A constant flow of seawater was maintained. Larvae were fed twice a day with a mixture

of *Brachionus* sp. and *Artemia* sp. *nauplii* (2040 individuals per 600 ml) and microalgae. During the first three days after hatching, decapsulated eggs of *Artemia* sp. were added to the mixture. Larvae were collected daily until metamorphosis.

Both eggs and larvae (after anesthetized with MS-222) were observed under a Nikon SMZ-800 stereomicroscope, photographed with a Nikon Coolpix 5400 camera and preserved in 4% saline formalin buffered with sodium borate. All larval measurements correspond to standard length.

In addition to embryonic and larval descriptions, some notes on the ontogeny of larval behaviour were taken, using the focal animal technique (Martin & Bateson, 1993). During each observation period, the occurrence of locomotory, non-directed and foraging behaviours (Barlow 1968) were recorded. Locomotory activities include swim and "pause-travel" behaviours (larvae scans for prey; if prey are not located the animal moves a short distance, stops, and scans again - similar to "saltatory-search", O'Brien, 1990); non-directed activities include pause (larva is motionless) and sink (larva is motionless and descends through the water column, often head first) behaviours; foraging includes orient (the head movement towards a prey item) and fixate (the larva is stationary and bends its caudal region into an "S" shape position) behaviours.

RESULTS

Both species lay the egg masses in a single layer underneath the rocks, with *L. purpurea* preferring larger stones. The male provides all parental care, funning and rubbing the eggs until hatching. For *L. purpurea*, mean batch area was 4.19 ± 1.36 cm^2 (mean ± S.D.; range = 1.94-5.18, n = 6), mean number of eggs per batch was 144.4 ± 46.46 eggs (mean

± S.D.; range = 60.14-177.1, n = 6) and egg density was 34.50 ± 3.39 eggs.cm^{-2} (mean ± S.D.;, n = 6); for *L. lepadogaster*, mean batch area was 4.61 ± 2.46 cm^2 (mean ± S.D.; range = 1.84-8.60, n = 7), mean number of eggs per batch was 123.1 ± 53.74 eggs (mean ± S.D.; range = 44.16-176.7, n = 7) and egg density was 27.71 ± 4.27 eggs.cm^{-2} (mean ± S.D.;, n = 7). Recently laid eggs of both species are bright yellow, becoming orange by the end of development. They are oval in shape, with a lower flattened surface containing fine filaments for attachment to the rocks (Fig. 1). Egg diameter is however significantly different between the two species (mean ± S.D. = 1.8 ± 0.04 mm, range = 1.7-1.9, n = 53 for *L. purpurea* and mean ± S.D. = 1.9 ± 0.03 mm, range = 1.8-1.9, n = 46 for *L. lepadogaster*; t-test: t = -2.35, df = 34, $p < 0.05$).

Embryonic development lasted 21 days in *L. purpurea* (mean water temperature ± S.D. = 14.2 ± 0.67º C, range = 13-15º C, n = 20) and 16 days in *L. lepadogaster* (mean water temperature ± S.D. = 16.5 ±0.46º C, range = 16-17º C, n = 16). The main ontogenetic events of the embryonic development of each species are shown in Table I. In the embryos of both species, the yolk sac was not segmented and had a large oil droplet, surrounded by several small droplets. Circulation of the blood fluid was first registered on day 9, in *L. purpurea*, and on day 6, in *L. lepadogaster*. Pigmentation begun to appear in the eyes, followed by pigmentation in the lateral side of the body. In *L. purpurea*, punctiform melanophores covered the middle region, spreading to the anterior and posterior region (cephalic and caudal area) changing into star shape melanophores throughout development. In *L. lepadogaster*, star shape melanophores covered both the lateral side of the body and the caudal region until the end of the myomeres, spreading to the cephalic region in the following days.

The major difference between the embryos of these two species is the absence or residual pigmentation in the ventral fin fold region of *L. purpurea* (Fig. 1A), that clearly contrast with the strong pigmentation composed by star shape melanophores in the ventral region of the gut in *L. lepadogaster* (Fig. 1B). Three star shape melanophores which become ramified until hatching are also present in the lower jaw of *L. lepadogaster* (Fig. 1B).

Table I - Ontogenetic events of embryonic development of *Lepadogaster purpurea* and *L. lepadogaster* in order of first appearance: [1] cell cleavage; [2] embryo recognizable; [3] cephalic and caudal dilatation; [4] embryo reaches the margin of the yolk; [5] notochord differentiation; [6] eyes; [7] brain; [8] eye lens; [9] myomeres; [10] pigmented eyes; [11] auditory vesicles; [12] otoliths; [13] brain lobes; [14] median fin fold; [15] tail bud free of the yolk; [16] gut differentiation; [17] heart beatings; [18] notochord; [19] embryo larger than the egg; [20] embryo movements; [21] anus differentiation; [22] mouth differentiation; [23] anus opened; [24] caudal region differentiation; [25] pectoral fin buds; [26] mouth visible but closed; [27] opercula visible but closed; [28] eye movements; [29] peristalsis; [30] liver differentiation; [31] mouth opened; [32] mandibles differentiation; [33] opercula opened.

	Event																																
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
L. purpurea																																	
13-15 º C	d1	d4	d5	d5	d5	d6	d6	d7	d7	d7	d7	d7	d8	d9	d10	d10	d10	d10	d10	d10	d11	d13	d14	d15	d15	d15	d15	d15	d16	d16	d17	d17	d20
L. Lepadogaster																																	
16-17 º C	d1	d3	d3	d3	d4	d3	d4	d4	d3	d4	d5	d5	d5	d5	d6	d6	d6	d7	d7	d6	d7	d8	d9	d11	d10	d9	d11	d10	d11	d11	d11	d12	d13
16-17 º C	d1	d4	d4	d4	d5	d4	d5	d4	d4	d5	d5	d5	d6	d5	d7	d5	d7	d7	d8	d7	d7	d8	d9	d10	d10	d9	d10	d10	d11	d11	d11	d11	d12

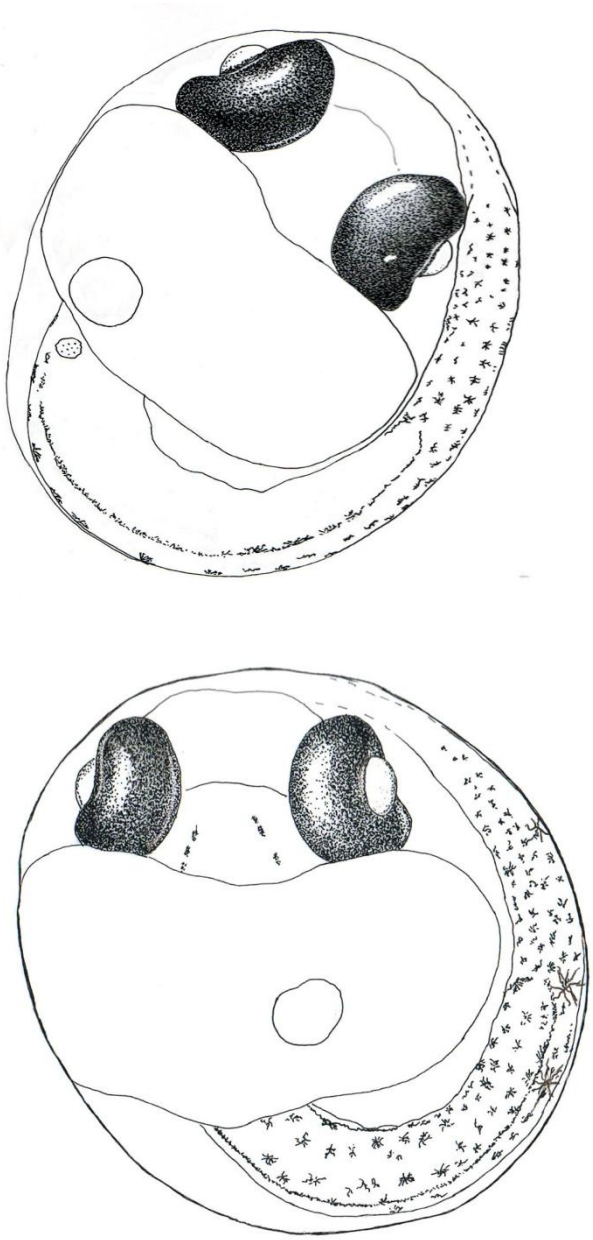


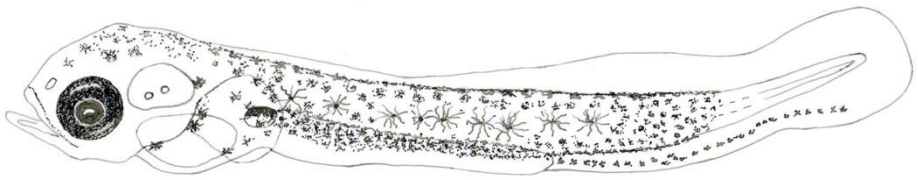
Fig.1. Dorsal view of the embryo of *Lepadogaster purpurea* (A) and *Lepadogaster lepadogaster* (B).

Near hatching, movements of the embryos increase, especially eye movements. Hatching of the entire batch occurred throughout a two-day period. Larvae of both species hatched with the head first and immediately swam to the surface where they seemed to gulp air, probably to fill the gas bladder. Figure 2 presents larvae of both species collected at

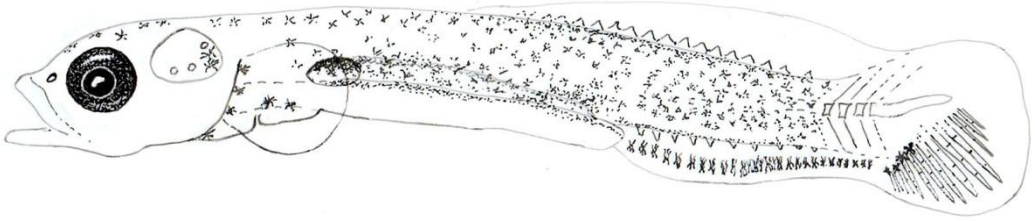
different developmental stages and the main ontogenetic events of larval development are shown in Table II.

Newly hatched larvae of both species measured 5.2 ± 0.08 mm (mean ± S.D.; range = 5.0-5.3 mm, n = 5 for *L. purpurea*; range = 5.2-5.3 mm, n = 5 for *L. lepadogaster*) and hatched with the mouth and anus opened, lips and jaws differentiated, eyes completely formed and fully pigmented, the nostrils opened and the yolk almost fully absorbed (Fig. 2). The opercula were open, with three branchial arches present. The characteristic nostril tentacles of adult fishes were not yet developed. The liver and the heart were completely formed and the blood circulation was noticeable. Larvae hatched with both pectoral fins differentiated but without any rays and with the median fin fold ranging from the cephalic area to the anus.

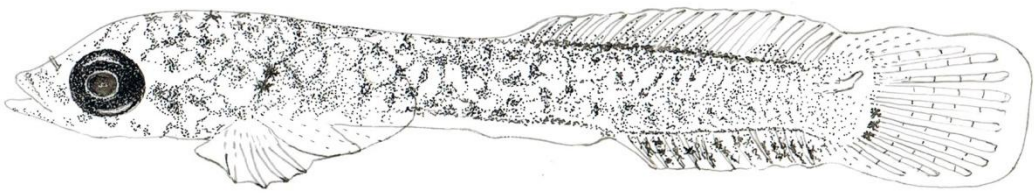
A-I



A-II



A-III



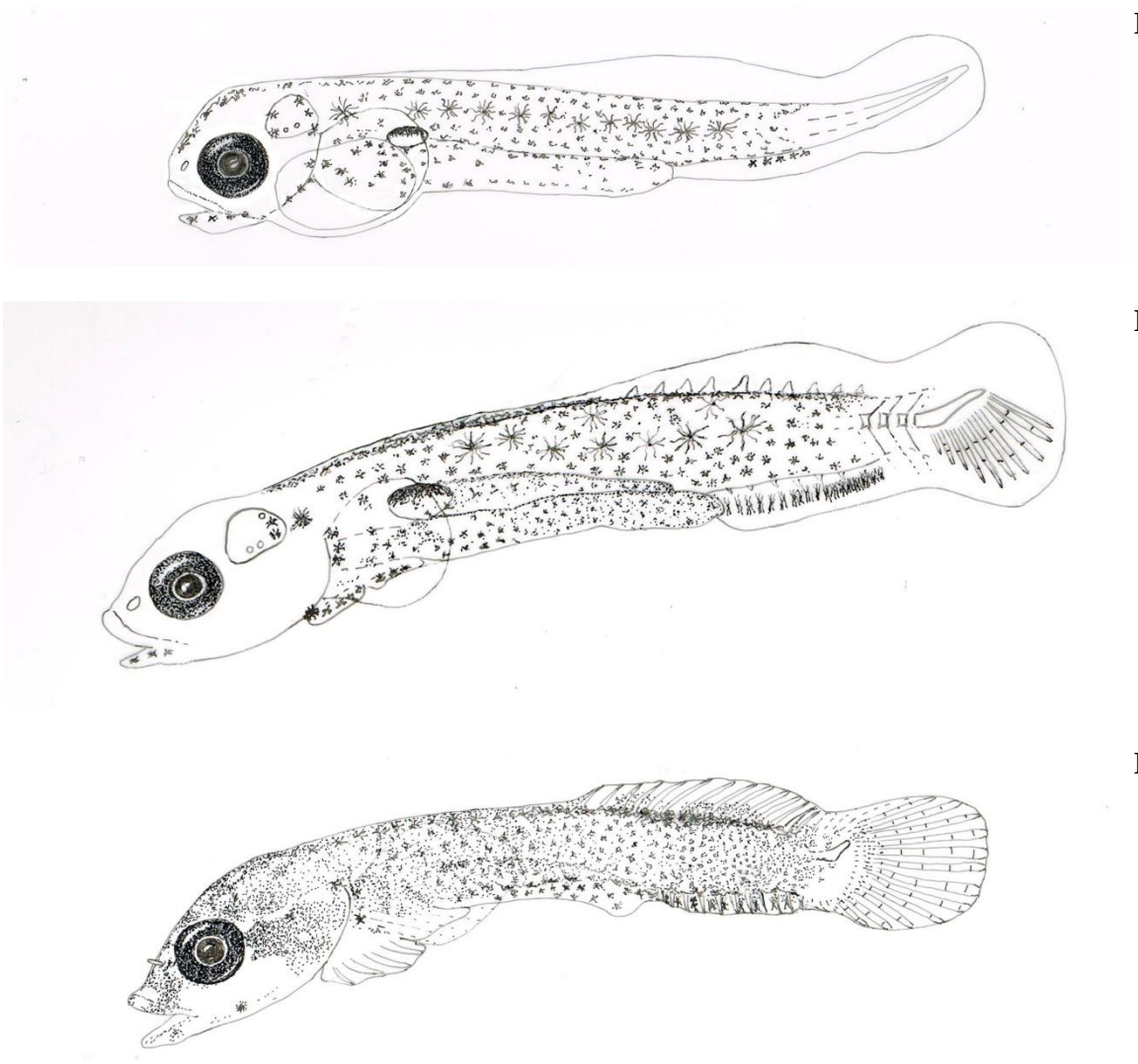


Fig. 2. Larvae of *Lepadogaster purpurea* (A) and *Lepadogaster lepadogaster* (B) collected at different development stages: (I) newly hatched larva, (II) post-flexion larva with the caudal fin rays differentiated and with anal and second dorsal fin rays starting to develop, (III) juvenile.

Table II- Ontogenetic events of larval development of *L. purpurea* and *L. lepadogaster* in order of first appearance (days after hatching): [1] filled gas bladder; [2] yolk absorption; [3] exogenous feeding; [4] caudal fin rays; [5] pectoral fin rays; [6] notochord starts to flex; [7] ventral disk differentiation; [8] larvae started to settle; [9] dorsal fin rays; [10] anal fin rays; [11] ossified vertebra; [12] teeth; [13] notochord flexion completed; [14] all larvae settled; [15] median fin fold reabsorption; [16] tentacles differentiation; [17] juvenile typical pigmentation. Size ranges are also included.

	Event																
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
L. purpurea																	
13-15 º C	d1	d2	d2	d8	d9	d12	d12	d13	d25	d25	d27	d30	d30	d33	d33	d39	d39
	5.4-7.3 mm			7.4-8.3 mm					8.4-9.3 mm					9.4 > 10 mm			
13-23 º C	d1	d2	d2	d9	d9	d11	d11	d13	d25	d25	d26	d26	d28	d27	d31	d32	d32
	5.4-7.3 mm			7.4-8.3 mm					8.4-9.3 mm					9.4 > 10 mm			
L. Lepadogaster																	
16-18 º C	d1	d1	d1	d4	d5	d6	d6	d7	d11	d11	d13	d14	d17	d18	d19	d20	d25
	5.4-6.3 mm			6.4-7.0 mm		7.1-8.3 mm						8.4-9.3 mm					
17-18 º C	d1	d1	d1	d5	d5	d6	d6	d10	d11	d11	d16	d17	d14	d15	d15	d15	d19
	5.4-6.9 mm			7.0-8.9 mm				9.0-9.9 mm			10.0-11.2 mm						

The pigmentation patterns were quite similar with a few exceptions and changed throughout development in the two species. Both larvae were strongly pigmented at hatching, with two parallel rows in the dorsal area composed of *ca*. 30 ramified melanophores in *L. purpurea* and 26 in *L. lepadogaster*, which cover the pre- and post-anal area until the last 4-6 myomeres. In the lateral trunk, ramified melanophores were distributed from the post-opercular area, forming a V-pattern coincident with the myomeres, until the last 4-6 myomeres. In the ventral trunk, there were *ca*. 12 ramified melanophores in *L. purpurea* and 8 in *L. lepadogaster*. Close to the urostyle, two punctiform melanophores were present in both species. On the ventral area of the median fin fold there were ca. 30 star-shape melanophores in *L. purpurea* and 21 in *L. lepadogaster*. The gut region was heavily pigmented on both the dorsal and lateral sides, with ramified melanophores. In *L. purpurea*, the anus was surrounded by melanophores and, by contrast, in *L. lepadogaster*, melanophores agglomerated near the anus but did not surround it. The gas bladder had a distinct dorsal pigmentation in both species. Similarly to what was observed for the embryos, the most noticeable difference between species was the absence or residual ventral pigmentation in the gut region of *L. purpurea*, while *L. lepadogaster* presented more than 30 ramified melanophores in the ventral region of the gut. In the base of the pectoral fin there were 2 melanophores in *L. purpurea* and 3 in *L. lepadogaster*. In the dorsal cephalic area, larvae of both species had 3 major sets of ramified melanophores organised in the following pattern (counting from the tip of the nose to the post-ocular area): 4+11+13 in *L. purpurea* and 4+9+7 in *L. lepadogaster*. In the median head, behind the opercula, there were ca. 10 ramified melanophores in both species. Inside the internal auricular vesicle, on a dorsal view, there were 2 melanophores visible in *L. purpurea* and 4 in *L. lepadogaster*. A single punctiform melanophore on the gular region, as well as 2 ramified melanophores in the throat

region, 4 in the inferior lip and 1 in the opercula were present in *L. lepadogaster* but absent in *L. purpurea*.

In *L. purpurea*, 3 ramified melanophores appeared in a row in the post-anal lateral side, above the notochord at day 4 (6.7-7.3 mm). These pigments disappeared later in development, at day 10 (7.8-8.3 mm). At day 12 (8.0-8.3 mm), the notochord started to flex, the ventral disk (modification of the pelvic fins) started to differentiate and the larval body becomes less pigmented, with the exception of the star-shape melanophores in the anal median fold and in the cephalic region (Fig. 2A-II). At day 33 (9.4-9.5 mm), all larvae were settled and acquired a benthic life style and all fin rays were formed: D = 17 (17-21); C = 11 (11-14); A = 11 (10-12); P = 21 (20-23). Larvae begun slowly to metamorphose and pigmentation started to become similar to the adult fish. By day 39 (9.5-10 mm) the nostril tentacles were already formed.

In *L. lepadogaster*, at day 6 (7.1-7.3 mm), coincident with the notochord flexion, a regression on the expansion of the melanophores in the lateral region was registered, and the ventral disc started to develop (Fig. 2B-II). At day 13 (8.0-8.3 mm), 7 ramified melanophores were noticeable in the anterior, medium and posterior area of the ventral disc region. Larva started to make contact with the bottom of the aquarium at day 7 and by day 18 (8.4-9.3 mm) all larvae were settled. At this time, all fin rays were formed: D=17 (17-21); C=12 (11-14); A=11 (10-12); P=21 (20-23) and the nostril tentacles begun to differentiate.

Juvenile pigmentation started to appear after settlement. In both species, the eyes were silver with some pale red and orange colours, the head was carmine with some whitish spots and the body was heavily pigmented with carmine-orange pigments. In *L. purpurea*, the ventral

region which lacked pigmentation, started to acquire a pinkish shade. Both species presented, in the cephalic region, 3 sets of melanophores (from the tip of the nose to the post-ocular area: 2+11+13) and in the base of the pectoral fin 3 ramified melanophores could be distinguished. In the base of the dorsal fin of *L. purpurea*, there were 3 melanophores between the 1st, 3rd and 5th rays; the caudal fin had 2 ramified melanophores between the 3rd and 5th rays and 1 ramified melanophore at the end of the notochord; a single melanophore in the anterior area of the sucking disc, 2 in the median area and 1 in the posterior area were characteristic at this stage. In *L. lepadogaster*, at day 21 (8.3-8.5 mm), 3 star shape melanophores were present in the caudal fin region. These were the same melanophores that were visible at the end on the notochord before flexion started. The dorsal region of the gut was heavily pigmented and in the anus opening there was a set of melanophores. In the ventral area of the gut, 2 rows of ramified melanophores were distinguished. Only the base of the dorsal and anal fins was pigmented with the anal fin presenting star shape melanophores. A single ramified melanophore in the inferior jaw was observed.

Swimming and foraging activities increased with ontogeny in both species, although *L. purpurea* was less active, spending more time in resting activities (pause and sink).

DISCUSSION

Eggs of both species have an oval shape with a flattened surface with fine filaments for attachment to the rocks. The mean length of the long axis of the eggs was 1.8 mm in *L. purpurea* and 1.9 mm in *L. lepadogaster*, which agrees with the available descriptions on other gobiesocids (Allen, 1984; Hefford, 1910; Padoa, 1956; Russel, 1976). However, Breining & Britz (2000) report smaller lengths for *L. lepadogaster*, ranging from 1.5 to 1.8 mm. According to several authors, the number of eggs per clutch can vary from 200 to 250

(Allen, 1984; Russel, 1976), or up to 300 (Padoa, 1956). Nevertheless, in this study, the larger clutch obtained in captivity had 177 eggs, for each species, with up to three different developmental stages recognized, which agrees with previous descriptions (Breining & Britz 2000). Eggs of the two species differ in the amount of pigmentation in the embryo, with *L. lepadogaster* presenting significantly more pigments especially in the mouth and ventral region of the gut.

The embryonic development of *L. purpurea* lasted 21 days at an average temperature of 14.2º C and 16 days in *L. lepadogaster* at 16.5º C. Larvae of both species hatched with the head first, after several movements of the trunk and head. The observed advanced developmental level at hatching is typical of marine fishes with male parental care (Thresher, 1984; Sponaugle *et al.*, 2002; Hickford & Schiel, 2003) that spawn demersal eggs. After hatching, larvae swam immediately to the surface, with successive swimming impulses, where they seemed to gulp air, likely to fill the gas bladder. This behaviour has also been reported in other demersal spawners, such as *Gobius paganellus* (Pisces: Gobiidae) (Borges *et al.*, 2003).

The change to a benthic mode of life was gradual in both species, with larvae increasingly spending more time close to the bottom until definitely settling. Larval development lasted 33 days in *L. purpurea*, at a mean temperature of 14.6º C, and 18 days in *L. lepadogaster*, at a mean temperature of 16.5º C. These values are in accordance with the estimated pelagic larval duration (PLD) described by Beldade *et al.* (2007) for *L. lepadogaster*, based on otoliths readings of new settlers collected in the field (mean ± S.D. = 14.9 ± 2.2, range = 11-18, n = 13). Although there are no field studies on the PLD of *L. purpurea*, our results suggest a much longer period, approximately 30 days. This difference is relevant for species that hatch with the same size and similar morphologies and may be explained by the striking

difference in the breeding periods of the two species. The lower water temperature in the winter probably increases developmental time of both eggs and larvae since it is well know that developmental time decreases with increasing temperature in many fish species (Blaxter, 1969).

Behavioural observations showed that *L. purpurea* was less active when compared to *L. lepadogaster* in terms of swimming and foraging behaviour, and spent more time in resting activity (pause and sink). This may be a consequence of the life history of *L. purpurea*. This species is a winter spawner, has a greater pelagic larval duration and is committed to being in the water column much longer than *L. lepadogaster*. During this extended larval period, larvae will likely encounter periods of low plankton availability. This condition probably requires a different strategy from *L. lepadogaster*. Spending time in locomotion and foraging activities is energetically costly to larvae (Kiorboe & Munk 1986). In the scope of the life history characteristics of *L. purpurea*, spending more time in pause and sink behaviours is likely to be a better strategy for saving energy. Faria & Gonçalves (*in press*) have studied routine and critical swimming behaviour of these species and have also seen that *L. purpurea* is a poorer swimmer than *L. lepadogaster*.

Both species changed from a saltatory strategy to a cruise strategy during development, more or less coincident with the beginning of notochord flexion. The pause-travel behaviour is probably associated to a saltatory strategy of searching for prey: the search for prey occurs only while pausing between swimming events (Browman & O'Brien, 1992a, b). As larvae became more developed, most of their time was spent swimming, and they adopted a cruise strategy: the search for prey occurs while swimming (Munk & Kiorboe, 1985). Additionally, foraging behaviour increased with development, which can be explained by an enhanced

swimming capacity and visual acuity, which in turn will improve encounter rates and feeding success (Miller et al., 1993).

The nature of the non-directed activities as sink and pause is not straightforward (Rabe & Brown, 2001). Sinking has been reported in other species, such as the snapper, *Pagrus auratus* (Pankhurst et al., 1991) and black sea bream, *Acanthopagrus schlegeli* (Fukuhara, 1987). Like the pause behaviour, it has been interpreted as a resting behavior. In this study, sinking behaviour was more common on early stages of *L. lepadogaster* and disappeared in settlement stage larvae, while *L. purpurea* exhibited this behaviour throughout the ontogeny, although more frequent during the early stages.

The two species can be differentiated at hatching by the pigmentation on the ventral region, which is absent in *L. purpurea*. As opposite, *L. lepadogaster* hatches with several pigments on the ventral region, from the lower jaw till the anus. Additionally, one pigment positioned on the tip of the lower jaw, four melanophores on each side of the lower jaw and two lines of melanophores on the ventral side of the gut till the anus are characteristic of *L. lepadogaster*. Later in development, pigmentation in the ventral sucking disk also allows distinguishing between the two species, given that *L. purpurea* has 4 pigments, whereas *L. lepadogaster* has 7 pigments. When larvae reach the juvenile stage, *L. purpurea* acquires a slightly darker and purple pigmentation when compared to *L. lepadogaster*. The absence or residual ventral pigmentation in *L. purpurea* is still a feature at this juvenile stage.

The only other species of the genus, *L. candolii*, is considerable less pigmented when compared to *L. lepadogaster* and *L. purpurea* (Guitel, 1888; Allen, 1984). The presence of only few pigments on the dorsal trunk and lateral trunk are the most obvious characteristics that allow differentiating among species of the same genus.

The available descriptions for northeast Atlantic and Mediterranean larvae of Gobiesocidae are clearly incomplete (see Guitel, 1888; Padoa, 1956; Russel, 1976). In particular, the two species described in this study have been mistakenly identified until very recently (Henriques *et al.*, 2002). Consequently, the few larval developmental studies of *Lepadogaster* available (Guitel, 1888; Padoa, 1956; Russel, 1976) were useless in identifying both species correctly, since the developmental stages were all mixed into a single species. For example, the descriptions made by Guitel (1888) for *L. purpurea* (previously considered as *L. gouanii* – see Henriques *et al*., 2002) were in fact larvae of *L. lepadogaster*. This can be verified by analyzing the available draws where the presence of ventral pigmentation and of pigments located on the inferior jaw, which are absent from *L. purpurea*, unmistakably ascribes these larvae to *L. lepadogaster*.

The correct identification of fish larvae is the basis for ecological and taxonomic studies of the pelagic stage of fishes (Leis & McCormick, 2002). Errors in identification can lead to misinterpretations of ecological processes (Powles & Markle, 1984). In particular, dispersal patterns are species specific with related species having different behavioural patterns that may affect their dispersal (Leis & McCormick, 2002).

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CHAPTER III. ONTOGENY OF LARVAL SWIMMING BEHAVIOUR

Ontogeny of swimming behaviour of two temperate clingfishes, *Lepadogaster lepadogaster* and *Lepadogaster purpurea* (Gobiesocidae)

Faria, A.; Gonçalves, E. (2010). Ontogeny of swimming behaviour of two temperate clingfishes, *Lepadogaster lepadogaster* and *Lepadogaster purpurea* (Gobiesocidae). *Marine Ecology Progress Series* 414, 237-248.

ABSTRACT

Gobiesocids are typically reef dwelling species and their larvae have been found in several nearshore rocky environments, which suggest a possible retention pattern for these species. Retention may occur due to physical features of the area and/or active larval behaviour, such as swimming abilities. In the laboratory, we measured the ontogeny of swimming behaviour (routine speed and critical speed [U_{crit}]) of two clingfish species, *Lepadogaster lepadogaster* and *L. purpurea*. *L. lepadogaster* larvae swam better than *L. purpurea*, but this difference may be related to differences in water temperature, since the former is a spring spawner, whereas *L. purpurea* spawns during the winter. It is well known that water viscosity increases with decreasing temperature, making swimming more difficult. Routine and critical swimming speeds of larvae of both species increased with ontogeny (size), even though variability at any ontogenetic state was high. Critical speed (U_{crit}) ranged from 1 to 9.4 cm s^{-1} in *L. lepadogaster* and from 1.2 to 6.5 cm s^{-1} in *L. purpurea*. Routine speeds were *ca* 18-19% of the maximum U_{crit} registered for both species. Interestingly, close to settlement size (10-11 mm TL) swimming speed started to decrease concurrently to the development of a ventral sucking disk which allows individuals to attach to the bottom of the swimming chamber and counteract strong currents. This shift in swimming behaviour associated to settlement is probably an adaptation to the cryptobenthic mode of life of these fish.

KEYWORDS: Gobiesocidae, swimming performance, ontogeny, retention, behaviour.

INTRODUCTION

Fish inhabiting nearshore reefs are valuable elements of coastal biodiversity, playing an important ecological role in the functioning of littoral ecosystems (Depczynski & Bellwood 2003, La Mesa et al. 2004). Current gaps in recruitment knowledge of marine species justify an increased effort aimed at a deeper understanding of the rocky coastal fish assemblages. The early stages of cryptobenthic fish ['small bodied fish (5-10 cm) that exploit restricted habitats where food and shelter are obtained in, or in relation to, conditions of substrate complexity and/or restricted living space, with a physical barrier likely to be interposed between the small fish and sympatric predators' (cf. Miller, 1979)], in particular, are still largely unknown, but some biological characteristics, such as a small pelagic larval duration (PLD) and large larval sizes at hatching, suggest that some species may be able to remain nearshore. Several studies of tropical and temperate reef fishes have found that larvae hatching from demersal eggs are more abundant nearshore, whereas those that are more widely distributed are generally derived from pelagic eggs (e.g. Leis & Miller 1976, Leis & Goldman 1987, Marliave 1986, Suthers & Frank 1991). Pelagic eggs are typically smaller than demersal eggs, and usually produce smaller larvae (Thresher 1984), with less-developed sensory systems and swimming abilities (Blaxter 1986, Miller et al. 1988, Fuiman 2002, Snelgrove et al. 2008) when compared to larvae hatching from demersal eggs. The PLD has also been proposed as one of the primary correlates of dispersal ability (Thresher et al. 1989, Sponaugle et al. 2002, Lester & Ruttenberg 2005), in that species with short PLD have potentially more limited dispersal (Swearer et al. 2002) than species with longer PLD (Bradbury et al. 2008).

Clingfish species (family Gobiesocidae) occur worldwide, in many different habitats in tropical and temperate seas (Briggs 1955, 1986). Although clingfish larvae have been collected in several studies in nearshore rocky environments (Marliave 1986, Kingsford

& Choat 1989, Brogan 1994, Tilney et al. 1996, Sabatés et al. 2003, Beldade et al. 2006a, Borges et al. 2007), knowledge of their larval behaviour and ecology is extremely poor. At the Arrábida Marine Park (Portugal), R. Borges & E.J. Gonçalves (unpublished data) caught large numbers of gobiesocid larvae of all size classes with light traps placed over the nearshore rocky reefs. In contrast, no larvae of these species (Borges et al. 2007) were collected in offshore sampling. These suggest nearshore retention of these fishes. As clingfish larvae begin to switch from a pelagic to a benthic environment, they develop a ventral adhesive disk, which is an adaptation to their cryptobenthic mode of life. Once larvae settle to a hard substrate, the individuals remain hidden underneath rocks their entire adult life (Gonçalves et al. 1998).

Retention of larvae near reefs is increasingly recognized as a central mechanism of self-recruitment for some coral reef populations (e.g. Jones et al. 1999, Swearer et al. 2002, Taylor & Hellberg 2003). One of the advantages of nearshore retention for coastal species is the ability to locate a suitable habitat at settlement. Dispersion may increase mortality because variable oceanographic processes influence larval transport, both temporally and spatially, and if larvae are not transported to an appropriate habitat they can be lost (Hickford & Schiel 2003). Retention and/or dispersal may depend on particular physical features of the area and/or active larval behaviour, such as swimming abilities. Recent *in situ* and laboratorial studies have shown strong swimming capabilities in coral reef fish larvae (e.g. Leis & Stobutzki 1999, Fisher & Bellwood 2002, 2003, Fisher 2005, Leis et al. 2009a, b), which are sufficient to influence oceanic dispersion and return to adult habitat (e.g. Leis & Carson-Ewart 1997, Stobutzki & Bellwood 1997). Besides swimming ability, larvae need also to detect suitable habitats at settlement, because it is unlikely that successful settlement is solely by chance (Jones et al. 1999, Cowen et al. 2002).

Studies on swimming performance of temperate fish larvae are few (e.g. Dudley et al. 2000, Clark et al. 2005, Leis et al. 2006a, Guan et al. 2008, Faria et al. 2009), relative to tropical reef environments (e.g. Stobutzki & Bellwood 1994, 1997, Fisher et al. 2000, 2005, Fisher 2005, Leis et al. 2007, 2009a, b). Moreover, most investigations have concentrated on late-stage larvae, and rarely consider the ontogeny of swimming behaviour (but see Clark et al. 2005, Guan et al. 2008, Faria et al. 2009, Leis et al. 2007, 2009a, b). Two of the most common measurements of swimming capability include routine speed and critical speed (U_{crit}). Routine swimming is generally considered to be important in foraging and provides critical information on the ability of larvae to influence their dispersal patterns, because it is an estimate of the undisturbed, "day-to-day" speeds of larvae (Fisher & Leis 2009). Critical swimming speed (U_{crit}) is a useful estimate of maximum swimming performance of fish larvae (Plaut 2001, Fisher 2005). Although critical swimming speed is a measure of prolonged swimming speed that is rarely, if ever, experienced by fish in nature (Plaut 2001), it provides a useful metric for comparing taxa or developmental stages (Leis 2006), and can be correlated with other more ecologically relevant measures of swimming ability, such as routine, *in situ* and endurance performance.

In this study we characterize the ontogeny of critical (U_{crit}) and routine swimming speed of two temperate Gobiesocidae species, *Lepadogaster lepadogaster* and *L. purpurea*. These closely-related species are quite similar in morphology, but differ in their breeding seasons (Henriques et al. 2002).

MATERIAL AND METHODS

Larvae. Larvae were obtained from breeding individuals maintained in separate 250 l aquaria. Females laid eggs on the underside of rocks placed in their aquarium, which were guarded by the male. As hatching approached, rocks with eggs were removed from the parental aquaria and transferred to a 30 l larval rearing tank, where larvae hatched. Aquaria were maintained at 14.5-15.5 ºC for *Lepadogaster purpurea* and 16-17 ºC for *L. lepadogaster*, which was consistent with the mean water temperature at the sampling area in the testing dates (Henriques et al. 2002). All tanks were maintained at a constant salinity of 34 PSU. Larvae were fed three times per day with *Brachionus* sp. enriched with Selco (Artemia systems), which was gradually replaced with *Artemia* sp. nauplii five days after hatching. Data were collected throughout ontogeny using more than one cohort of larvae in the same day (6 cohorts for *L. lepadogaster* and 5 cohorts for *L. purpurea*).

Critical swimming speed (U_{crit}). U_{crit} was measured using a swimming chamber, following the protocols of Stobutzki & Bellwood (1994, 1997). The chamber was made of clear Perspex with 6 parallel swimming lanes, each 30 mm wide, 50 mm high and 180 mm long. A removable lid allowed introduction and removal of fish from the lanes. A strip of black tape on the top of the lid provided fish with a visual reference to maintain position in the flow, and mesh screens at the upstream and downstream ends of each lane retained larvae in the chamber. Flow straighteners (40 mm long)at the upstream end of each lane minimized turbulence. Previous work demonstrated that water velocity was not significantly different between the centre of the lane and 5 mm from the wall for typical U_{crit} values (Stobutzki & Bellwood 1997, Stobutzki 1998, Fisher et al. 2000). Experimental observations also confirmed that larvae had no depth preference in the

chamber. The swimming chamber was part of a closed flow system in which submersible pump moved water from a collecting tank to the swimming chamber. A ball valve at the upstream end of the swimming chamber controlled water velocity. A protractor mounted on the valve handle calibrated flow rates in swimming lanes based on handle angle; for different angles we recorded the time taken for the outlet water to fill a 5-l container and divided by the cross-sectional area and number of lanes. Flow speed for a specific valve angle was averaged from three trials to form a calibration curve that formed the basis for setting flow velocities for experimental runs.

The different breeding seasons for the two species necessitated that experiments be conducted at different times of the year*: Lepadogaster lepadogaster* was tested during the spring-summer months (May to July) and *L. purpurea* was tested in the winter months (December to March). Larvae were tested every two days from hatch to settlement for each batch (Table 1). We tested 5-6 fish during the morning of each experimental day. One hour after feeding, larvae were carefully removed from the rearing tank and placed individually in large Petri dishes to acclimatize for 1 h undisturbed (Fuiman & Ottey 1993). After this period, larvae were transferred to the swimming chamber, one larva per lane, and maintained for 5 min at a flow speed of 1 cm s^{-1}. The few individuals that displayed symptoms of stress, such as lying on the bottom or clinging to the sides, after this acclimation period, were removed and replaced. Chamber water temperatures varied from 14.9 to 15.4 ºC (15.1 ± 0.15) for *L. purpurea* experiments, and from 16.4 to 17.5 ºC (16.9 ± 0.34) for *L. lepadogaster* experiments.

To measure U_{crit}, we increased water velocity by approximately 1.2 cm s^{-1} every 2 min until the larva was unable to swim against the current for 2 min. Calculation of U_{crit} followed Brett (1964):

$U_{crit} = U + (t/t_i * U_i)$,

where U is the speed of the penultimate increment, Ui is the velocity increment, *t* is the time swum in the final velocity increment, and t_i is the time interval for each velocity increment (2 min). After the test, fish were immediately photographed under a dissecting microscope and returned to the rearing aquarium.

This procedure was adopted because these species spawn few large eggs at a time and larvae are resilient to manipulation and can be kept alive through ontogeny. This strategy increases the likelihood of re-testing the same larvae, but there were between 20 to 60 larvae present in the aquarium at any given time, depending on the tested batch, and the probability of resampling the same individual was low. Closer to metamorphosis, resampling was more likely because numbers were lower. Interspecific comparison of the same developmental stage is unaffected by any re-testing and different batches were used to increase the robustness of the derived trends. Therefore, we are confident that resampling did not affect the outcome and interpretation of this study.

Routine swimming speed. The routine swimming test measured the mean rate of travel for individual larvae during undisturbed activity in the absence of food. Experiments were conducted throughout the larval period, from hatching to settlement, following Fuiman et al. (1999). Five larvae of similar size were removed from the rearing tank the day prior to testing. Each of the five larvae was then placed into a separate glass bowl (15 cm diameter, opaque black sides) in 1 l of filtered sea water at the same temperature and salinity as their rearing tank. Before each experiment, white paper was placed below the bowl to increase contrast. All but approximately 200 ml (1 cm depth) of water was siphoned carefully from the watch bowl to reduce measurement error caused by any

change in depth by the larva. The larva was left undisturbed for 2 min, after which its behaviour was recorded for a 2 min period. All five larvae were tested four times per individual. Larval behaviour was measured later through frame-by-frame analysis of video recordings. Routine swimming speed was determined for each larva by measuring the total distance covered in the 120 s recorded. After the test, fish were immediately photographed under a dissecting microscope, and returned to the rearing aquarium.

Data analysis. We examined the relationship between swimming performance (U_{crit} and routine speed) and total length (TL) by regressing swimming speed against size. Both the dependent and independent variables were $\log_{10}$ transformed to normalize data. To determine the best predictor of performance, values of critical and routine swimming speed were regressed against TL using linear, power, logarithmic and exponential models. The linear model produced the highest R^2, and was therefore adopted. For each species multiple regressions tested relationships between the two independent variables, age and size, and critical speed.

Analysis of covariance (ANCOVA) determined whether the slopes of the regressions of swimming performance on size differed among cohorts, with U_{crit} and routine speed as the dependent variable, cohort as a fixed factor, and size as co-variate. ANCOVA also tested whether slopes of regressions of swimming performance on size differed among species, with U_{crit} and routine speed as the dependent variable, species as a fixed factor, and size as co-variate.

Throughout ontogeny, changes in body size influence swimming speed by placing larvae in different hydrodynamic regimes. Reynolds number (Re) is a measure of the ratio of

viscous to inertial forces as fish swim through water that it is commonly used to characterize different hydrodynamic conditions. Re (Webb & Weihs 1986) was calculated for critical swimming experiments to determine whether larvae were in viscous (Re < 200) or inertial (Re > 200) conditions, using the formula:

$Re = U * TL / v$,

where U is critical speed, TL is total length and v the kinematic viscosity of sea water (viscosity of sea water at 20ºC = 1.03×10^{-6} m^2 s).

The threshold of 200 is the most conventionally used number (Webb & Weihs 1986), and although recent experiments indicate that the viscous environment could extend to values of Re = 300, and a fully inertial environment may not come into play until Re > 1000 (e.g. Fuiman & Batty 1997), these thresholds are also species-specific. Because we lack information for *Lepadogaster* spp., we chose to use the most conventional and conservative value of Re.

For progressive stages of development, we measured TL to the nearest 0.01 mm, using Image J software (version 1.38). Before each photograph was taken, a transparent acetate sheet marked with a millimetre grid was photographed and used as reference before each measurement in the image analysis software. We used STATISTICA software (StatSoft, Inc., Version 6.0) for all statistical analyses.

RESULTS

Mean larval size (TL) on day 1 (when they were first tested) did not differ between species (*L. purpurea* mean size = 6.3 mm, SD = 0.53, range = 5.4-7.5, N = 20; *L. lepadogaster* mean size = 6.0 mm, SD = 0.49, range = 5.2-6.9, N = 14; t-value = 1.80, df

= 32, p = 0.08). Growth rates were also not statistically different between species (*L. purpurea* mean growth = 0.27 mm d^{-1}, SD = 0.58; *L. lepadogaster* mean growth = 0.19 mm d^{-1}, SD = 1.04; t-value = 0.26, df = 29, p = 0.80).

(1) Critical swimming speed

A total of 154 larvae of *L. lepadogaster* and 139 larvae of *L. purpurea* were tested for critical swimming tests. Of these, 9 recently hatched larvae of *L. lepadogaster* and 6 recently hatched larvae of *L. purpurea* could not swim at the slowest tested current speed, and 11 post-settlement larvae of *L. lepadogaster* did not swim at the maximum current speed because they had developed a ventral adhesive disc and attached to the bottom of the chamber. These individuals were excluded for all subsequent analysis and are omitted from Table 1.

Table 1. Summary of measurements of critical swimming speed (cm s^{-1}) and routine swimming speed (mm s^{-1}) for *Lepadogaster lepadogaster* and *Lepadogaster purpurea*: Number (N), size (total length – TL), age (dph: days post hatching), stages of reared larvae (Pre: preflexion; F: flexion; Post: postflexion) and number of tested cohorts.

	N	TL (mm)	Age (dph)	Stages	Cohorts
Critical speed (cm s^{-1})					
L. lepadogaster	134	5.1-10.9	0-19	Pre, F, Post	6
L. purpurea	133	5.3-10.9	1-21	Pre, F, Post	5
Routine speed (mm s^{-1})					
L. lepadogaster	82	5.1-10.9	2-19	Pre, F, Post	6
L.purpurea	42	6.2-10.4	2-21	Pre, F, Post	5

The U_{crit} values ranged from 1 to 9.4 cm s^{-1}, corresponding to 1.5 to 9.6 body lengths (BL) s^{-1} for *L. lepadogaster*, over a size range of 5.1 to 10.9 mm TL (Table 1). As expected, U_{crit} increased with size ($F_{(1,132)}$ = 75.90, p < 0.0001) (Table 2, Fig. 1). In *L.*

purpurea, U_{crit} also increased significantly with size ($F_{(1,131)} = 283.60$, $p < 0.0001$) (Table 2, Fig. 1) from 1.2 to 6.5 cm s^{-1} (2.1 to 6.8 BL s^{-1}) over the size range tested (5.3 to 10.9 mm TL) (Table 1). In all cases, there was large variation in performance among individuals at any ontogenetic stage (Fig. 1).

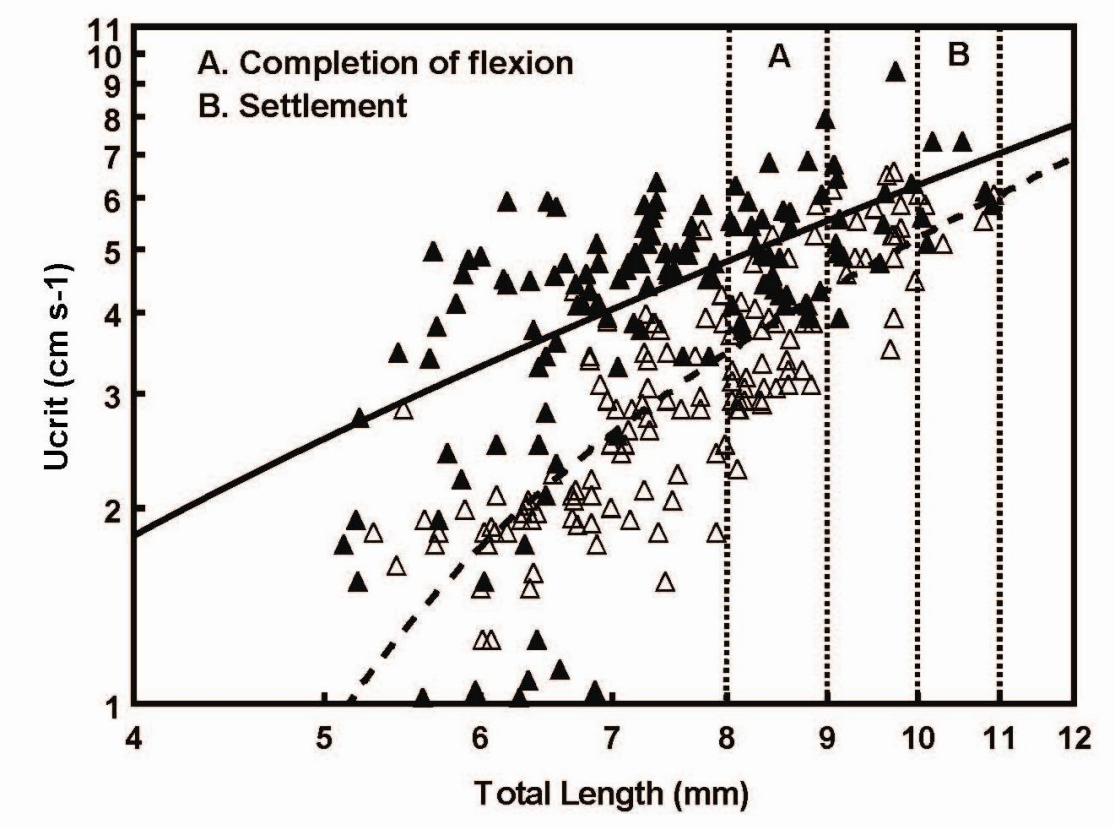

Fig 1. Ontogenetic trend for critical swimming speed of *Lepadogaster lepadogaster* (black symbols) and *L. purpurea* (white symbols) larvae. Each symbol represents the test result for an individual larva. The thick line is the regression line for *L. lepadogaster* and the broken line is the regression line for *L. purpurea* (see regression details in Table 2). Both axes are log-transformed to reflect developmental progress. Vertical bars represent size at which two important developmental milestones are attained: A. completion of notochord flexion and; B. settlement.

In both species, critical speed increased with size over the common range tested, with an increase of 0.5 to 1.6 cm s^{-1} per mm growth for *L. purpurea* and 0.3 to 1.2 cm s^{-1} per mm growth for *L. lepadogaster*. Incremental speed increase was significantly greater for *L.*

lepadogaster ($F_{(1, 9)}$ = 32.42 , p < 0.0005). Multiple regression analyses were significant for both species (*L. purpurea*: $F_{(2, 130)}$ = 244.55, p < 0.0005, adjusted R^2 = 0.79; *L. lepadogaster*: $F_{(2, 131)}$ = 56.17, p < 0.0005, adjusted R^2 = 0.45). Age and size were both good predictors of critical speed, although age was better (Table 3).

Table 2. Relationships between critical speed (U_{crit}: log_{10} transformed) and size (Total Length – TL: log_{10} transformed), U_{crit} and age (dph: days post hatch), routine speed (log_{10} transformed) and size (TL: log_{10} transformed) and routine speed and age (dph). See Table 1 for number, size and age range of tested larvae. CI = confidence interval, ns = not significant (p > 0.05).

	Relationship	**R^2**	**p**	**Slope ± 95% CI**
***U_{crit}* (cm s^{-1}) *vs* size**				
L. lepadogaster	y = 1.582x – 0.764	0.37	<0.001	1.582±0.359
L. purpurea	y = 2.049x – 1.332	0.68	<0.001	2.049±0.241
***U_{crit}* (cm s^{-1}) *vs* age**				
L. lepadogaster	y = 0.028x + 0.389	0.44	<0.001	0.028±0.005
L. purpurea	y = 0.028x + 0.263	0.76	<0.001	0.028±0.003
Routine (mm s^{-1}) *vs* size				
L. lepadogaster	y = 0.804x + 0.0004	0.05	ns	0.804 (0.61-0.92)
L.purpurea	y = 1.275x – 0.640	0.06	ns	1.275±1.61
Routine (mm s^{-1}) *vs* age				
L. lepadogaster	y = 5E-05x + 0.714	< 0.01	ns	5E-05±0.010
L.purpurea	y = 0.009x + 0.412	0.02	ns	0.009±0.019

Table 3. Multiple-regression models for the relationship of critical swimming speed with two independent variables (age and size).

	Model *F*-value	**Model adjusted R^2**	***p*-value**	**beta**
L. purpurea	244.55	0.79	< 0.0005	
Age			< 0.0005	0.59
Size			< 0.0005	0.33
L. lepadogaster	56.17	0.45	< 0.0005	
Age			< 0.0005	0.45
Size			0.004	0.27

Some differences in performance among cohorts were apparent for both *L. lepadogaster* ($F_{(5,127)}$ = 5.00, p = 0.0003) and *L. purpurea* ($F_{(4,127)}$ = 5.71, p = 0.0003), indicating that the slopes of each cohort regression of U_{crit} on size were not homogenous and hence the influence of size on U_{crit} differed among cohorts (Fig. 2). This cohort variation might be the result of differences in size structure among cohorts or performance differences. ANCOVA comparisons between species found a significant interaction effect (factor * co-variate: $F_{(1,264)}$ = 111.35, p < 0.0001), indicating that the influence of size on U_{crit} differed between species, with *L. lepadogaster* a significantly better swimmer than *L. purpurea* (Fig. 1).

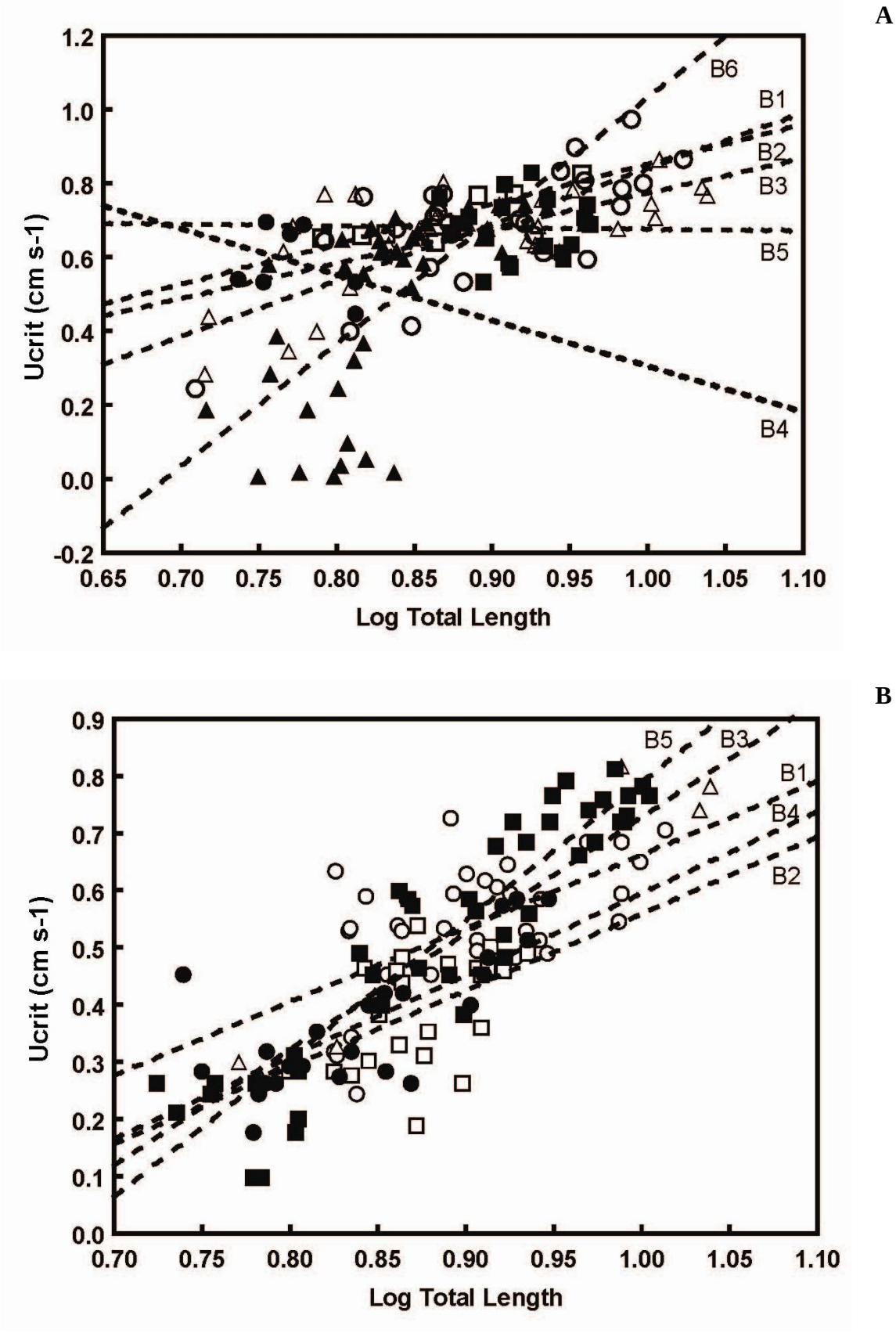

Fig 2. Ontogenetic trend for critical swimming speed of each batch of *Lepadogaster lepadogaster* (A) and *L. purpurea* (B) larvae. Each symbol represents the test result for an

individual larva. Both variables are log-transformed. Each line represents the regression lines of each batch: *L. lepadogaster* B1 (open circles): y = 1.51x – 0.67; B2 (open squares): y = 1.08x – 0.23; B3 (open triangles): y = 0.95x – 0.18; B4 (closed circles): y = - 1.24x + 1.54; B5 (closed squares): y = - 0.04x + 0.72; B6 (closed triangles): y = 3.32x – 2.29; *L. purpurea* B1 (open circles): y = 1.29x – 0.62; B2 (open squares): y = 1.34x – 0.78; B3 (open triangles): y = 2.03x – 1.30; B4 (closed circles): y = 1.43x – 0.84; B5 (closed squares): y = 2.41x – 1.63.

Re increased as individuals developed from hatch to juveniles (Fig. 3). However, *L. purpurea* Re was smaller compared to *L. lepadogaster* as a result of slower critical swimming speeds. Before the beginning of notochord flexion (TL < 7 mm), individuals of both species were clearly within a viscous hydrodynamic environment (Re < 200). During notochord flexion, larva's swim performance improved, and after completion of flexion (8 to 9 mm) individuals swam in an inertial environment.

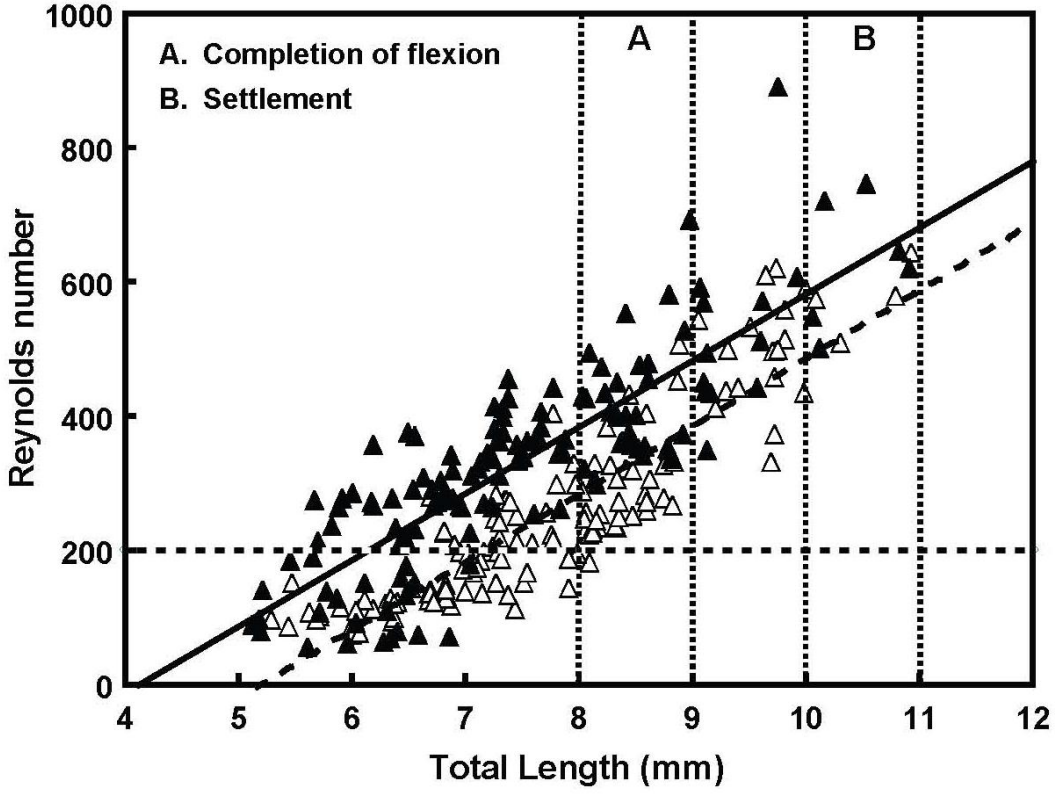

Fig 3. Reynolds number (Re) for larvae of *L. lepadogaster* (black symbols) and *L. purpurea* (white symbols). The horizontal dotted line at Re = 200 represents the transition from a viscous to an inertial environment. The thick line is the regression line for *L. lepadogaster* and the

broken line is the regression line for *L. purpurea*. Vertical bars represent size at which two important developmental milestones are attained: A. completion of notochord flexion and; B. settlement.

(2) Routine swimming speed

We tested a total of 95 larvae of *L. lepadogaster* from six cohorts, and 49 larvae of *L. purpurea* from five cohorts for routine swimming experiments. Of these, 13 *L. lepadogaster* larvae and 7 *L. purpurea* larvae did not swim since they were either recently hatched or settled and they were excluded from the analysis.

For both species there was a non-linear relationship between routine swimming speed and size (*L. lepadogaster*: $F_{(1,80)} = 2.85$, $p = 0.09$; *L. purpurea*: $F_{(1,40)} = 2.57$, $p = 0.12$) (Table 2, Fig. 4). Despite large variation in routine speed at any given size, swimming performance increased with development in *L. lepadogaster*, until around 9 mm TL, after which it decreased (Fig. 4). Routine swimming speed varied from 0.8 to 17.8 mm s^{-1} (0.1 to 2 bl s^{-1}) over a size range of 5.1 to 10.8 mm (TL) for *L. lepadogaster*, and from 0.1 to 11.5 mm s^{-1} (0.02 to 1.6 bl s^{-1}) over a size range of 6.2 to 10.4 mm (TL) for *L. purpurea* (Tables 1, 2). When testing for between-cohort differences in routine swimming in each species, there was no significant effect of $\log_{10}$TL (*L. lepadogaster*: $F_{(1,75)} = 2.62$, $p = 0.11$; *L. purpurea*: $F_{(1,36)} = 1.53$, $p = 0.22$), no significant interaction (*L. lepadogaster*: $F_{(1,75)} = 0.15$, $p = 0.70$; *L. purpurea*: $F_{(1,36)} = 0.42$, $p = 0.52$) and no significant differences in routine swimming among cohorts in each species (*L. lepadogaster*: $F_{(5,75)} = 0.78$, $p = 0.57$; *L. purpurea*: $F_{(4,36)} = 0.39$, $p = 0.81$). However, ANCOVA revealed a significant effect of $\log_{10}$TL ($F_{(1,121)} = 5.07$, $p = 0.03$) and significant difference in routine swimming behaviour between the two species ($F_{(1,121)} = 19.25$, $p<0.0001$).

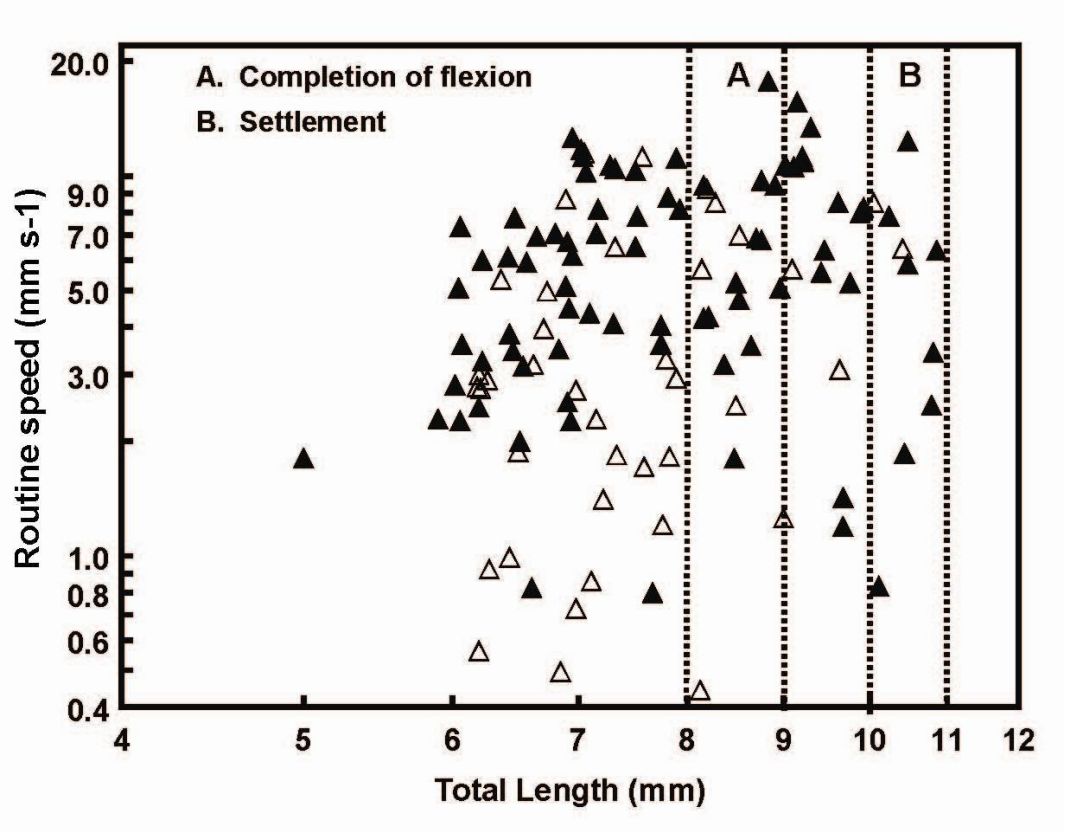

Fig 4. Relationship between routine swimming speed and total length (TL) for *Lepadogaster lepadogaster* (black symbols) and *L. purpurea* (white symbols). Each symbol represents the mean of four replicates on an individual fish. Both axes are log-transformed to reflect developmental progress. Vertical bars represent size at which two important developmental milestones are attained: A. completion of notochord flexion and; B. settlement.

DISCUSSION

Swimming abilities of larval fish are critical behavioural traits because swimming influences the capacity of larvae to find food, escape from predators, and influence dispersal (Stobutzki & Bellwood 1994, 1997). In order to determine the potential importance of swimming behaviour, it is fundamental to know how abilities change during ontogeny. Most research has however focused on swimming abilities of late-stage larvae (e.g. Stobutzki & Bellwood 1997, Fisher & Bellwood 2002, Fisher & Wilson 2004,

Fisher et al. 2005), although there are a few recent studies that included smaller pre-settlement larvae (e.g. Fisher et al. 2000, Clark et al. 2005, Leis et al. 2006a, b, 2007, Guan et al. 2008, Faria et al. 2009, Leis et al. 2009a, b). Nevertheless, few incorporate the ontogeny of swimming behaviour.

The two gobiesocids studied here are important cryptobenthic components of the north-eastern Atlantic temperate rocky reefs (Beldade et al. 2006a). The larval stages of these fishes are poorly known compared to their tropical counterparts with respect to behavioural traits and ecology (Leis et al. 2006a, 2009a, Faria et al. 2009). In fact, most temperate studies have focused on clupeiform, gadiform, or pleuronectiform larvae (e.g. Blaxter 1986, Miller et al. 1988), in contrast to the perciform species that are superior swimmers and compromise most tropical studies (e.g. Fisher et al. 2000, 2005, Leis & Fisher 2006). Additionally, at all size classes, tropical perciform reef fish larvae feed and swim more efficiently with more developed sensory apparatus than other larval fishes (Leis & McCormick 2002). Data on temperate reef fish larvae are therefore needed to address this data.

At a given size, *L. lepadogaster* swam faster than *L. purpurea* and increased swimming capacity at a greater rate. This is surprising given that these closely-related species have similar morphologies, life-history trajectories, larval growth rates, size at hatching and rocky reef habitat, though *L. purpurea* shelters in larger boulders at greater depths than *L. lepadogaster* (Henriques et al. 2002). They differ, however, in timing of breeding (*L. lepadogaster* spawns from March/April to June/July and *L. purpurea* spawns from October/November to March/April, Henriques et al. 2002). Larvae of the two species therefore experience different thermal environments because mean winter temperatures

vary from 14.5-16 ºC whereas spring temperatures vary from 18-19.5 ºC (Henriques et al. 2002).

Temperature can affect swimming performance of fish larvae in two ways. On the one hand, fish muscle cells operate more efficiently at higher temperatures (Hunt von Herbing 2002). On the other hand, temperature is necessarily linked to viscosity effects on fish larval motion (Podolsky 1994, Fuiman & Batty 1997, Hunt von Herbing 2002). Whether larvae swim in a viscous or an inertial environment has a major effect on their swimming performance because the interaction between larvae and water in viscous environments makes swimming energetically expensive (Hunt von Herbing 2002). Recently hatched larvae of both species swim in a viscous environment (Re < 200). During notochord flexion, larvae improve their swimming abilities, and after notochord flexion is complete, individuals swim at speeds that place them outside a strictly viscous hydrodynamic environment (Re > 200). Nevertheless, *L. purpurea* had smaller Re than *L. lepadogaster*. Given that these species hatch at a similar size, and water viscosity in winter is only 5% higher than in summer, differences in Re are explained by the slower critical swimming speed of *L. purpurea*. To determine the relative importance of physical and physiological factors on swimming performance of larvae of these species, further studies are needed that quantify swimming kinematics at different temperatures, viscosities, and different sizes. In addition, there are a range of other biological and physiological parameters that may influence swimming performance and could explain both intra and inter-specific differences in swimming performance (e.g. growth rate, Kolok & Oris 1995; allometric growth, Muller & Videler 1996; dietary fatty acid composition, McKenzie et al. 1998; proportion of red-muscle, Koumoundouros et al. 2009), but these variables are beyond the scope of the present study.

In both gobiesocids, we found a roughly linear increase in critical speed with growth over a similar size range, with an increase of 0.5 to 1.6 cm s^{-1} per mm growth for *L. purpurea* and 0.3 to 1.2 cm s^{-1} per mm growth for *L. lepadogaster*. These increments are lower than speed increments reported for other pelagic, warm-temperate species (1.2 to 2.6 cm s^{-1} per mm growth, Clark et al. 2005), which may be related to temperature and/or morphological or taxonomical differences. Guan et al. (2008) concluded from their study of ontogenetic changes in critical speed of shorthorn sculpin, that temperature played an important role in the development rate of critical swimming speed as a function of both age and size. They also hypothesized that differences in critical swimming speed in cold-water species are mainly related to hatching size and the degree to which water temperature influences development of each species. As in other studies, critical speed varied substantially at any given size (Fisher et al. 2000, 2005, Clark et al. 2005, Leis et al. 2007, Faria et al. 2009). Although size is usually a better predictor of U_{crit} than age (e.g. Clark et al. 2005, Leis et al. 2007, Faria et al. 2009), we found the reverse pattern in these clingfishes, even though both factors were good correlates of U_{crit}. Critical swimming speeds (1 to 9.4 cm s^{-1} for *L. lepadogaster* and 1.2 to 6.5 cm s^{-1} for *L. purpurea*) were within the lower range of critical speeds reported for other temperate species (*e.g.* 1 to 25 cm s^{-1}, Clark et al. 2005), and are considerably lower than critical speeds reported for tropical reef fish larvae (11.3 to 61.5 cm s^{-1}, Leis & Fisher 2006). Critical speeds expressed in terms of body length (bl s^{-1}) ranged from 1.5 to 9.6 bl s^{-1} for *L. lepadogaster* and 2.1 to 6.8 bl s^{-1} for *L. purpurea*, which are also lower than speeds reported for tropical species (10 to 20 bl s^{-1}, Leis 2006).

Routine speed also increased through development, as seen in other studies (e.g. Fuiman et al. 1999, Fisher & Bellwood 2003). The average routine speeds measured were

however low, with larvae maintaining speeds of only *ca*. 18-19% of their U_{crit} (0.8 to 17.8 mm s^{-1} for *L. lepadogaster*, and 0.1 to 11.5 mm s^{-1} for *L. purpurea*). When relative swimming speeds (body lengths per second - BL s^{-1}) are considered, the values reported in the present study (0.02-2 BL s^{-1}) are well within the range of values reported for other temperate species (e.g. Blaxter 1986, Miller et al. 1988, Fisher et al. 2007). Still, routine swimming speed values reported for both tropical and subtropical species (Fuiman et al. 1999, Fisher & Bellwood 2003) are considerably higher than the ones described here.

These results are consistent with the general perception that temperate species are poorer swimmers than tropical species (Stobutzki & Bellwood 1997), but caution is needed when extrapolating between systems because there are no comparative data available for gobiesocids. Moreover, U_{crit} is a laboratory measure of forced performance and it is unlikely that larvae are able to sustain maximum critical speeds in the wild for extended periods (Leis 2006). Therefore, it is important to relate U_{crit} to other swimming measurements that are more applicable in the field, such as sustained swimming speed and *in situ* swimming speed. Sustained swimming speed is a measure of the maximum long-term swimming abilities of larvae and their capacity to influence dispersal over extended periods of time. For instance, Fisher & Wilson (2004) reported that settlement-stage larvae of nine tropical species could maintain a speed of ~ 50% U_{crit} for at least 24 hours in the laboratory. *In situ* speed is measured in the field by observing larvae released and followed by divers, and has the advantage that larva's swim speeds are actually measured in the ocean. Leis & Fisher (2006) and Leis et al. (2006a) concluded also that *in situ* speed was about half of U_{crit} in settlement-stage larvae of 83 species of coral-reef fishes of 11 families, and around 35-50% over a range of developmental stages (5-12 mm SL) in larvae of three warm-temperate demersal fish species. This relationship can be

used to estimate swimming speed of larvae in the ocean from laboratory measurements. Moreover, U_{crit} correlates with routine swimming speed. Fisher & Bellwood (2003) concluded that routine speed was ~ 20% of U_{crit} in three species (Apogonidae and Pomacentridae). Overall, U_{crit} is a useful measure of maximum swimming speed that is comparable across taxa and closely correlated with other ecologically relevant measures of swimming performance such as routine speed (Fisher et al. 2005), but extrapolations requires caution.

The large between species differences in routine swimming speeds might be explained not only by differences in temperature and in the swimming capacity of a given species, but also by the methodologies used. Methodological differences are one of the biggest difficulties in comparing routine measures of swimming speed. Factors such as tank size and volume, food distribution, starvation, light conditions, turbulence, and variability in individual behaviour, could influence swimming speed of larvae. For example, turbulence is known to increase feeding activity (McKenzie & Kiorboe 1995), lower food densities increase the time spent swimming (e.g. Munk & Kiorboe 1995) and undisturbed swimming speeds are greater at night than during the day (Fisher & Bellwood 2003). Furthermore, the external stimuli present in the natural environment but absent in the laboratory may mean that routine speed measured in laboratorial conditions are conservative relative to natural routine swimming speeds (Fisher & Leis 2009). These facts collectively suggest the need to standardize methods used to measure undisturbed swimming speeds of fish larvae to maximize cross-study comparisons.

Both critical and routine speeds increased steadily with size and age until settlement (*ca*. 10 mm); beyond this, they decreased. The lack of swimming (in the case of critical speed)

or reduced swimming (in the case of routine speed) of larvae close to settlement is probably related to behavioural changes associated with a benthic lifestyle, and not to decreased larva's swimming ability. These individuals were completing metamorphosis and resembled newly settled fish, including the presence of a ventral adhesive disk. Other studies have related changes in swimming performance to developmental or ecological transitions. In a study of sustained swimming abilities of New Zealand reef fish larvae, Dudley et al. (2000) reported that *Upeneichthys lineatus* (Mullidae) did not swim after settlement into a benthic life style. Guan et al. (2008) found that critical swimming speed improved steadily through ontogeny in shorthorn sculpin (*Myoxocephalus scorpius*) until metamorphosis, after which it slowed down. These authors also suggested that this decrease was linked to a habitat shift associated with settlement. In contrast, Fisher et al. (2000) found that coral reef fish larva's swimming ability increased slowly in early larval stages and faster in later stages, and Clark et al. (2005) found that swimming performance of four species of temperate marine fishes (Sciaenidae, Sparidae, Percichthyidae) improved markedly after notochord flexion was complete. Faria et al. (2009) also reported that critical swimming ability of red drum larvae increased more rapidly during the pelagic (pre-settlement) stage, during radical changes in body shape and structure.

Whether average swimming speeds recorded for the clingfishes reported here are sufficient to influence their position on reefs is unclear. Numerical circulation models indicate that only modest speeds (1 to 10 cm s^{-1}) are required to affect dispersal significantly (e.g. Pepin & Helbig 1997, Wolanski et al. 1997, Porch 1998). Additionally, active larval behavior can effectively reduce the dispersal of a number of taxa with varying pelagic larval durations (PLD). Reef fish larvae with relatively short PLDs (e.g. pomacentrids with PLDs of 24–29 d) may remain near source populations (Jones et al.

1999) using simple vertical migration (Paris and Cowen 2004). However, fishes with longer PLDs may also recruit back to source populations (Swearer et al. 1999), suggesting similar interactions with other variables. Guan et al (2008) described an unusual swimming behaviour of shorthorn sculpin in which larvae oriented their bodies slightly upward during critical swimming tests. This behaviour was repeatedly observed and may contribute to relevant changes in the position of larvae in the water column although no definite conclusions were drawn. Larvae also may be able to reduce dispersal by remaining within the benthic boundary layer where flow is considerably reduced (Breitburg 1991, Breitburg et al. 1995). Post-flexion or late-stage larvae of fishes spanning several temperate and tropical families have been collected in or near the benthic boundary layer (e.g. Leis 1986, Breitburg 1991, Kaufman et al. 1992, Beldade et al. 2006b). Shanks et al. (2009) found that for many taxa with larvae that remain in the benthic boundary layer, dispersal distance is less than that predicted based on PLD. Pelagic larval duration of *L. lepadogaster* varies between 11 and 18 days (Beldade et al. 2007), which is a relatively short PLD for a temperate rocky reef species (Raventós & Macpherson 2001). Although no information is available for *L. purpurea*, laboratory rearing suggests that the PLD might be slightly longer, close to 25 days (unpublished results).

Multiple factors may similarly favour retention of gobiesocids in nearshore environments. Both study species produce demersal eggs that hatch into comparatively large, actively swimming larvae with functional eyes and developed fins and guts. Within our study area (the Arrábida Marine Park, Portugal), Beldade et al. (2006b) found that *Lepadogaster* spp. larvae were largely confined to the near bottom layer in all stages of development, a pattern confirmed in light trap studies(R. Borges et al., unpublished results). Moreover,

no larvae of these species were collected in offshore sampling (Borges et al. 2007). The advanced morphological development at hatch might facilitate active swimming to reduced-flow regions neat the bottom (Breitburg 1991, Breitburg et al. 1995) and therefore avoid dispersal (Black & Moran 1991, Black et al. 1991).

Taken together, these results indicate that clingfish larvae remain nearshore during the entire pelagic larval stage, most likely near the bottom and they may be particularly will adapted to these environments. Indeed, the slow speeds, near-bottom distribution of all developmental stages and short PLD's suggest that these species do not require strong swimming abilities because they do not disperse far from natal reefs. Further studies of self-recruitment and genetic differentiation in these species are needed, however, to address these issues. Finally, caution is needed in extending inferences from laboratory-reared larvae because performance of these larvae may differ from wild larvae. Learned behaviours, such as avoidance, have been shown to differ between wild and reared individuals because larvae from these two have very different experiences (Olla et al. 1998, Brown & Laland 2001). Therefore, measurements of larval swim speeds from the wild are needed to infer how laboratory measurements may be extrapolated to understand ecological and life history patterns (Leis 2006). Further studies are needed of swimming modes of fish larvae (e.g. sustained swimming), in order to determine how they relate to U_{crit} and routine speed. Knowing when and if directed swimming takes place and under what circumstance, is essential for a better understanding of how behaviour influences larval recruitment patterns and processes.

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Ontogeny of Critical Swimming Speed of Wild-Caught and Laboratory-Reared Red Drum Larvae (*Sciaenops ocellatus*)

Faria, A; Ojanguren, A; Fuiman, L; Gonçalves, E (2009). Ontogeny of Critical Swimming speed of wild-caught and laboratory-reared larvae of Red drum (*Sciaenops ocellatus*). *Marine Ecology Progress Series* 384, 221-230.

ABSTRACT

Critical swimming speed (U_{crit}) provides a useful estimate of maximum swimming performance for fish larvae which can be used to assess transport and migratory potential. We measured U_{crit} of red drum larva (*Sciaenops ocellatus*) through its ontogeny and compared the swimming performance of laboratory-reared larva to that of wild-caught individuals. U_{crit} increased with ontogeny (size), even though variability in U_{crit} at any ontogenetic state was large. U_{crit} for wild-caught larvae increased from 9.7 to 22.2 cm s^{-1} over the range of 8.3 to 16.3 mm TL, and from 1.1 to 20.5 cm s^{-1} over the range of 3.0 to 19.1 mm TL for reared larvae. The ontogenetic increase in critical swimming speed occurred in two phases – an early phase of rapid improvement and a later phase of slower improvement. This sharp change in the trajectory of swimming performance coincided with important changes in ecology, morphology, and hydrodynamics. During the early phase, larvae were pelagic, their growth was highly allometric especially in the caudal region, and they swam in the inertial hydrodynamic regime. The onset of the later phase coincided with settlement into seagrass beds, isometric growth, and inertial effects on locomotion. Wild larvae generally exhibited greater values of U_{crit} than reared larvae of a comparable size, but the difference was not statistically significant. The results of this comparison imply that research on reared larvae may provide naturalistic results for swimming performance and that hatchery-produced larvae may perform certain behaviors well when released into the wild.

KEYWORDS: scaling, ontogeny, swimming performance, settlement, hydrodynamics, wild larvae, reared larvae

INTRODUCTION

Studies on the behavioural abilities of late-stage pelagic larvae have shown that some species are unexpectedly strong swimmers and have high endurance (Stobutzki & Bellwood 1994, Leis & Carson-Ewart 1997). As they approach settlement stage, larvae of many fish become "effective" swimmers (*sensu* Leis & Stobutzki 1999), meaning that they are able to swim faster than mean ambient current speeds (Stobutzki & Bellwood 1994, Leis & Carson-Ewart 1997, Fisher et al. 2000, Jenkins & Welsford 2002, Trnski 2002, Leis & Carson-Ewart 2003). These abilities have the potential to influence survival by affecting the capacity of larvae to avoid predators, find food, and control dispersal (Stobutzki & Bellwood 1994, 1997).

Studying behavioural ontogeny requires sampling larvae representing a wide variety of ontogenetic stages (sizes). Late-stage larvae of some species are easily captured in good condition using light traps (Doherty 1987) or fixed nets (Dufour & Galzin 1993). Usually, younger larvae can only be captured using plankton nets and are more susceptible to injury or death in the process (Leis et al. 2006b). For that reason, studies of younger larvae have focused on species that can be reared in captivity (Leis et al. 2006b). An obvious and important question is whether the behaviour, or specifically swimming performance, of reared larvae is similar to that of wild larvae. From the available data, the answers are mixed. It is generally agreed that behaviours with a learned component, such as anti-predator behaviour, will differ between wild and reared individuals because larvae from the two sources have very different experience (Olla et al. 1998, Brown & Laland 2001). However, some tested behaviours present contradictory results. For example, reared fish may swim faster, slower, or they may have performances equivalent to wild fish, with ontogenetic variations further complicating this picture (von Westernhagen &

Rosenthal 1979, Danilowicz 1996, Smith & Fuiman 2004). The same is true for other behaviours. So, when using results from reared individuals, it is desirable to compare the behaviour of these larvae to that of wild fish, especially if the results will be used to make inferences about fish in nature (Leis 2006).

Some fishes that breed in coastal marine waters use estuaries as nursery habitat for their late-stage larvae and juveniles. To enter an estuary, larvae may rely on passive transport (Jenkins et al. 1997, 1999) or some form of active behaviour, such as selective tidal stream transport (Forward et al. 1998, 1999) or active swimming (Trnski 2002, Leis & Carson-Ewart 2003). Once in the estuary, high levels of swimming performance may be required both to prevent larvae from being advected out of the estuary and to find and settle in a suitable habitat (Montgomery et al. 2001).

Red drum (*Sciaenops ocellatus*) is an estuarine-dependent species that supports an important recreational fishery throughout the Gulf of Mexico and the southeastern United States. Red drum spawns during early autumn in coastal waters near passes and inlets where the pelagic eggs and larvae are carried by tides and currents into shallow bays and estuaries (Holt et al. 1989). After tidal transport into estuaries and initial settlement in habitats near inlets (at ca. 6 to 8 mm), juvenile red drum are thought to disperse throughout the estuary, with documented movements into shallow tidal creeks and lower salinity habitats (Peters & McMichael 1987, Stunz et al. 2002). Such dispersal can be passive (Jenkins et al. 1997, 1999) or an active behavioural response (Leis et al. 1996, Stobutzki & Bellwood 1997).

Critical swimming speed (U_{crit}) provides a useful estimate of maximum swimming performance of fish larvae (Plaut 2001, Fisher 2005). Although it is a measure of

prolonged swimming speed that is rarely, if ever, experienced by fishes in nature (Plaut 2001), it provides a useful measure for comparing taxa or developmental states (Leis 2006). Work on U_{crit} in fish larvae has concentrated on late-stage larvae of coral reef fishes, nearing settlement (e.g. Stobutzki & Bellwood 1997, Fisher & Bellwood 2002, Fisher & Wilson 2004, Fisher et al. 2005). However, to determine the potential importance of swimming behaviour, it is essential to know how these abilities change during ontogeny. Relatively few measures of swimming performance are available for larvae smaller than those at settlement stage, but there are a few recent studies that focus on that issue (*e.g.*, Fisher et al. 2000, Clark et al. 2005, Leis et al. 2006a, b, Leis et al. 2007, Guan et al. 2008).

The goals of our study were to describe the ontogenetic changes in swimming ability (defined as U_{crit}) of a warm temperate perciform species, *Sciaenops ocellatus*; and to compare swimming performance of wild-caught and reared larvae.

MATERIAL AND METHODS

Larvae. Red drum eggs were obtained from captive broodstock at the University of Texas Marine Science Institute's Fisheries and Mariculture Laboratory. Spawning was achieved without hormone injections by manipulating temperature and photoperiod. Eggs were collected within 10 h of spawning, placed into conical rearing tanks with approximately 40 l of filtered sea water and aeration and were left to hatch. Temperature and salinity were maintained at 25 to 27 ºC and 27 to 30 PSU, respectively. Rotifers (*Brachionus* sp.) were enriched with algae (*Schizochytrium* sp., Algamac 2000) for 45 min and then added to culture tanks daily to maintain densities of 5 to 10 rotifers ml^{-1} from day 1 to day 12 posthatching. When larvae were 10 days old, brine shrimp (*Artemia* sp.) nauplii, enriched overnight, were also added daily to culture tanks at densities of 10

ml^{-1}. In conjunction with this diet shift, tank volume was increased to 50 l. Ten percent of the total volume of water was exchanged daily in each tank. Throughout rearing and experimentation, larvae were maintained on a 12 h light : 12 h dark photoperiod. Tested larvae were randomly selected from six separate spawns that occurred between 10 September and 11 October 2007.

Wild red drum larvae were collected from seagrass meadows in the Aransas Bay, mostly from the southern end of Lydia Ann Channel, near Port Aransas, Texas, (97º 2'41.63'' W, 27 º 54'50.63'' N) between 25 September and 16 October 2007 (five collections, one per week). At collection sites, temperatures and salinities ranged from 27 to 29 º C and from 16 to 24 PSU, respectively. Newly settled larvae (size range = 8.3 – 16.3 mm total length [TL]) were collected using a 1-mm mesh plankton net towed behind a benthic sled. The rectangular opening of the net measured 1 m (width) x 0.25 m (height), and was towed slowly by hand to avoid damaging larvae. Collected larvae were placed in a bucket of ambient sea water with aeration and transported to the laboratory. Wild larvae were then placed in plastic tanks with artificial seagrass to reduce stress and filled with filtered sea water maintained at a mean (± S.D.) temperature and salinity of 27.2 ± 0.3 ºC and 26.7 ± 1.4 PSU. Larvae were fed *Artemia* nauplii and allowed to acclimate in the lab for approximately 48 h, by which time they readily consumed the *Artemia* provided.

Swimming chamber. The swimming chamber was used following the protocols of Stobutzki & Bellwood (1994, 1997). The chamber was made of clear Perspex with 6 parallel swimming lanes, each 30 mm wide, 50 mm high and 180 mm long. A removable lid allowed introduction and removal of fish from the lanes. A strip of black tape on the top of the lid provided fish with a visual reference to maintain position in the flow, and a mesh screen was placed at the upstream and downstream ends of each lane to retain

larvae. A section of flow straighteners, 40 mm long, was placed at the upstream end of each lane to minimize turbulence. Previous work demonstrated that at the typical U_{crit} water velocity was not significantly different between the centre of the lane and 5 mm from the wall (Stobutzki & Bellwood 1997, Stobutzki 1998, Fisher et al. 2000). Experimental observations also confirmed that larvae did not show depth preference in the chamber.

The swimming chamber was part of a closed system of water flow. A submersible pump (Ecovort 510, 330 Watt) moved water from a collecting tank located 65 cm below the swimming chamber. Water flowed out of the swimming chamber and into the collecting tank. A ball valve at the upstream end of the swimming chamber was used to control water velocity. A protractor was mounted on the valve handle, and the angle of the handle was calibrated to flow rates in the swimming lanes by recording the time taken for the outlet water to fill a 5-l container and dividing by the cross-sectional area and number of lanes. The average of three calibrations was used as the flow speed for a specific valve angle. A calibration curve was established at the start of the experiment, with the angle as the predictor and the water velocity as the dependent variable. This curve allowed us to determine the angles required to set velocities during the trials. Experiments were conducted using flow speeds in the lanes ranging from 1 to 22 cm s^{-1}.

Experimental protocol. Experiments with reared fish were performed throughout the larval period (over the size and age range of 2.8 – 19.1 mm TL and 9 - 31 days posthatching [dph], respectively). Larvae reared from six spawns were used so that batch effects could be tested. In the morning of each experimental day, 6 to 12 fish were tested. One to 2 hours after feeding, larvae were carefully removed from the rearing tank using a

small container and placed individually in large glass bowls with approximately 1 l of sea water and left undisturbed for 2 h to allow recovery from handling (Fuiman & Ottey 1993). For wild fish, 12 to 18 individuals from each collecting day were tested. Wild larvae were placed individually in the large glass bowls during the night and left undisturbed in a controlled temperature room for testing the next morning.

Six fish were transferred from the glass bowls to the swimming chamber, one in each lane, and allowed to acclimate for 5 min at a flow speed of 1 cm s^{-1}. If any behavioral symptoms of stress, such as lying on the bottom or clinging to the sides, were observed after this acclimation period, the individual was removed and replaced by another fish. Water temperatures in the chamber over the study period varied from 24 to 26 ºC.

To measure U_{crit}, water velocity was increased by approximately 1.5 cm s^{-1} every 2 min until the larva was unable to swim against the current for 2 min. Calculation of U_{crit} followed Brett (1964):

$$U_{crit} = U + (t/t_i \cdot U_i),$$

where U is the speed of the penultimate increment, U_i is the velocity increment, t is the time swum in the final velocity increment, and t_i is the time interval for each velocity increment (2 min). After the test, fish were euthanized by thermal shock and immediately photographed under a dissecting microscope. Notes on ontogenetic progress were made and TL was measured to the nearest 0.01 mm, using Image J software (version 1.38).

Data Analysis. Six spawns of reared larvae and five collections of wild larvae yielded measurements of U_{crit} from 338 and 87 larvae, respectively. The relationship between U_{crit} and TL was examined for each group of wild and reared larvae by analysing scatterplots and computing linear regressions of logU_{crit} against logTL, described by the equation:

(1) log*U*crit = $a + b \cdot$ (logTL)

where *a* and *b* are constants. All data were $\log_{10}$ transformed to homogenize variances and normalize the data. Relationships between logU_{crit} and age were also investigated for reared larvae for which age was known.

Twenty three wild larvae and nine reared larvae were able to swim at the highest speed that could be achieved in the chamber (22.2 cm s^{-1}) and could not be fatigued by the experimental protocol. Therefore, the actual critical swimming speed of these individuals was greater than 22.2 cm s^{-1}. As the percentage of wild larvae that could not be fatigued was appreciable (26%), Tobit regression models (Tobin 1958, Amemiya 1984) were used to obtain realistic slopes and intercepts for the relationships between logU_{crit} and logTL. The Tobit model is a modified linear regression, very efficient for estimating the relationship between an explanatory variable and truncated or censored dependent variable. This procedure accounted for right- censored data, i.e. individuals that were still swimming at the maximum speed that the swimming chamber could produce. Since ANCOVA doesn't take into acount censored data, the 95 % confidence intervals for the estimated slopes and intercepts (*a* and *b*, derived from the Tobit regression) were used to test for differences between batches of domestic fish and between capture dates and cohorts of wild caught larvae, and to compare swimming performance between wild and

reared larvae. For this latter analysis, we only considered reared larvae larger than the smallest fish collected from the wild. Chi-square model was used to evaluate the significance of the relationship between logU_{crit} and logTL. For non-censored data (batches 1, 3, 4, 5 and 6 of reared fish), the ordinary least-squares model was used.

The linear regression of logU_{crit} on logTL, for reared larvae, yielded a trend in the residuals, indicating that the log-linear model was not appropriate for those data. Thus, a piecewise regression model was used of the form:

(2) $\log U_{crit} = a + b \cdot \text{logTL} + c \cdot (\text{logTL} - d) \cdot (\text{logTL} > d)$,

where a is the intercept for the lower piece, b is the slope of the lower piece, c is the change in slope for the upper piece, and d is the breakpoint between the two pieces of the regression. The final term in the equation is a logical statement and takes on a value of 1 if true and 0 if false. If the 95 % confidence interval (CI) for parameter c includes 0 (zero), the piecewise model is rejected in favor of a linear model (equation 1).

Using unpublished morphometric data available for red drum, it was possible to explore the relation between changes in body shape and swimming capacity, particularly before and after the breakpoint determined by the piecewise regression model. A growth gradient for red drum larvae was constructed using the methods of Fuiman (1983) to identify the degree of differential growth in different parts of the body. This method uses the allometric equation:

(3) $y = b \cdot x^k$,

where *b* is a constant, *k* is the growth coefficient, *y* is the length of a body section , and *x* is a standard that represents size. In this case, eye diameter was chosen as the standard for size because it is a distinct organ that usually grows at a constant differential rate with respect to total length (i.e., it displays a single growth stanza; Fuiman 1983). Measurements of body sections were made parallel to the longitudinal axis of the fish, from the tip of the snout to a vertical line through each designated landmark (Fig.1). Lengths of body sections were measured on a developmental series of specimens. The growth coefficient (*k*) and its variability for each segment were determined as the slope and its 95 % CI in the regression of log-transformed data. The location of the body segment midpoint, the indepedent variable of the growth profile, was estimated by its mean position from the tip of the snout, as a percentage of TL.

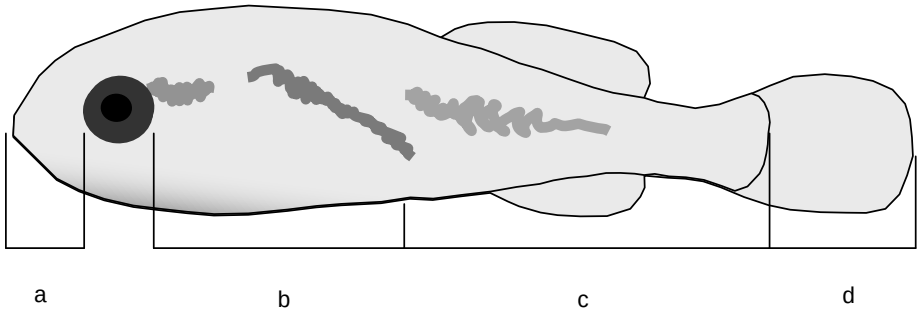

Fig 1. Measurements of body sections (*a*, *b*, *c* and *d*) of the larvae. a = snout length; b = pre-anal length, excluding the snout and the eye; c = post-anal length, excluding caudal fin; d = caudal fin length.

Changes in body size will also influence swimming speed by placing larvae in different hydrodynamic regimes. The Reynold's number (*Re*) is the index commonly used to characterize hydrodynamic conditions, and is calculated from the length and speed of a swimming organism and the kinematic viscosity of the surrounding fluid (Webb & Weihs

1986). *Re* was calculated to determine whether larvae were swimming at speeds where either viscous (Re < 600 for red drum larvae) or inertial forces (Re > 1300) predominate (Sarkisian 2005).

Statistical tests were conducted using SPSS (version 16.0) and Stata (version 10.0).

RESULTS

A total of 338 reared larvae from 6 spawns and 87 wild larvae from 5 weekly collections were tested. Of these, 23 wild larvae and 9 reared larvae could not be fatigued (i.e., U_{crit} was greater than 22.2 cm s^{-1}). Swimming performance (U_{crit}) increased with ontogeny (logTL), even though variation was large along the range of sizes tested (Fig.2). Critical speeds for wild larvae ranged from 9.7 to 22.2 cm s^{-1} over the size range of 8.3 to 16.3 mm (TL). This corresponds to length-specific speeds of approximately 9 to 21 body lengths (BL) s^{-1}. Swimming performance of reared larvae ranged from 1.1 to 20.5 cm s^{-1} over the range of 3.0 to 19.1 mm TL, corresponding to 3 to 20 BL s^{-1} (Table 1). For reared larvae, age was a poorer predictor of U_{crit} than size. Although all spawns showed a significant relationship between logU_{crit} and age, age explained less of the variation in logU_{crit} than logTL in five of the six spawns.

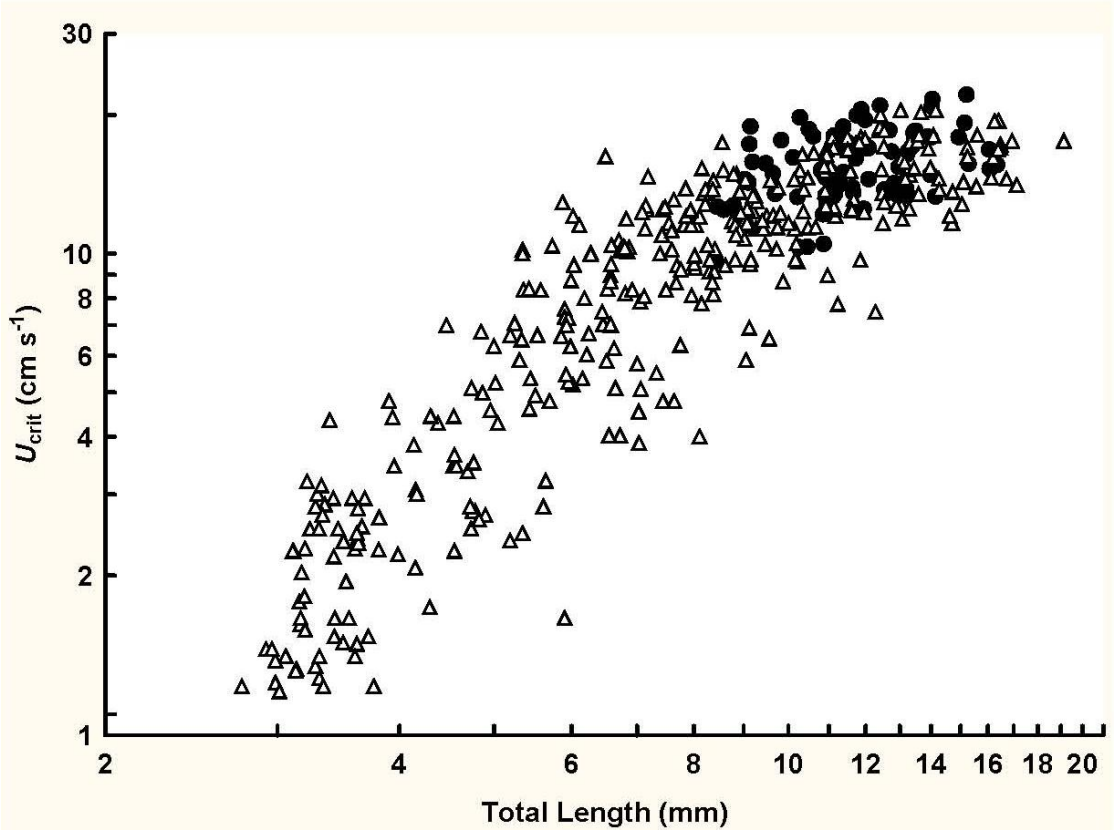

Fig 2. Ontogenetic trend for critical swimming speed of red drum. Each symbol represents results for an individual larva. White triangles represent laboratory-reared larvae and black circles represent wild-caught larvae.

Table 1. Summary of measurements of swimming performance for wild-caught red drum larvae (all collections combined) and six batches (B1 – B6) of red drum larvae reared from captive spawned broodstock. SD = standard deviation; nd = data not available.

				U_{crit} (cm s^{-1})		U_{crit} (BL s^{-1})	
	N	TL (mm)	Age (dph)	Range	Mean ± SD	Range	Mean ± SD
Wild larvae	87	8.3-16.3	nd	9.7-22.2	15.7 ± 2.7	9.4-20.7	13.6 ± 2.45
B1	26	10.2-15.3	20-24	7.5-20.5	16.0 ± 3.3	6.1-16.2	12.8 ± 2.6
B2	92	5.9-19.1	17-31	1.6-19.5	11.7 ± 3.8	2.7-25.1	11.6 ± 3.6
B3	115	2.8-14.2	9-27	1.1-20.5	8.6 ± 5.13	3.1-20.4	11.2 ± 4.6
B4	36	3.9-13.4	12-17	4.4-20.0	10.0 ± 4.1	6.5-17.7	11.6 ± 3.3
B5	40	3.1-6.3	10-14	1.5-11.6	4.2 ± 2.6	4.3-18.9	9.2 ± 3.6
B6	29	3.0-6.6	10-13	1.3-9.5	4.0 ± 2.1	3.7-15.7	8.4 ± 3.1

Although wild larvae seemed to have slightly better swimming performance (Fig.3), Tobit regression showed there was no difference between trends for wild and reared larvae. Comparisons of the 95 % CI revealed no significant differences in slopes and intercepts of the two regression models (Table 2).

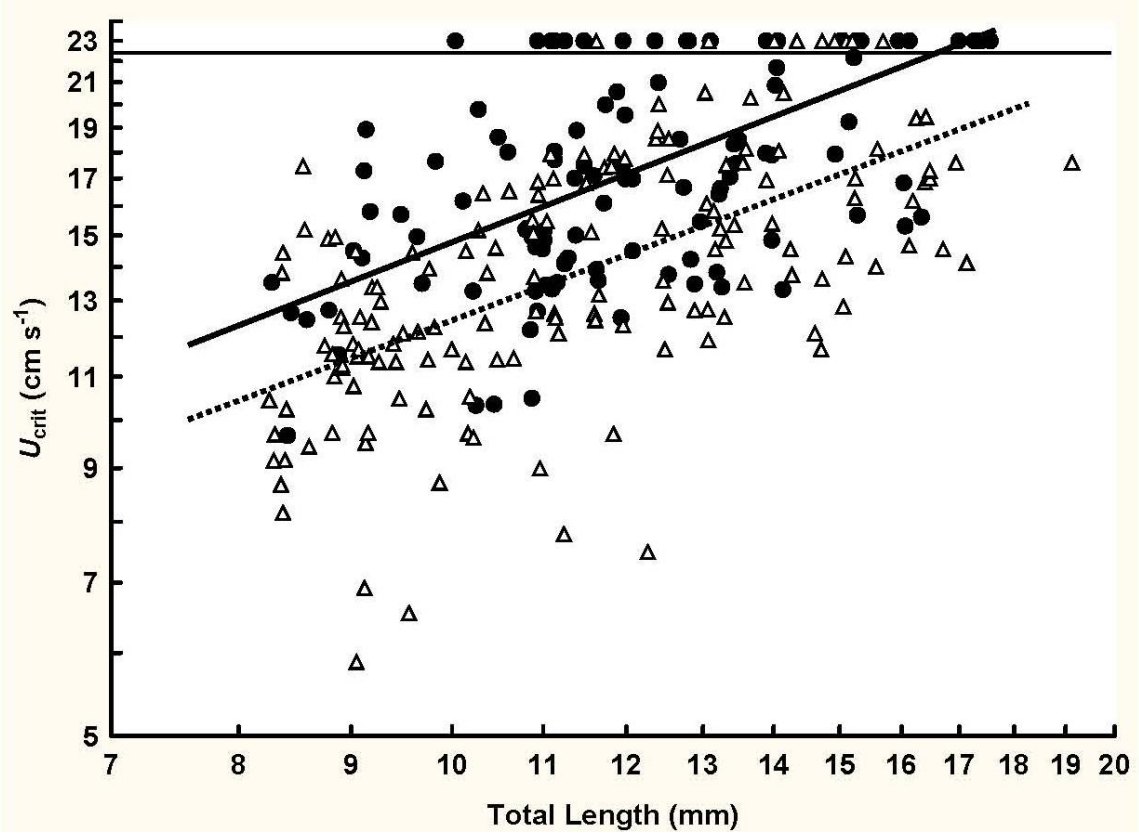

Fig 3. Relationship between critical swimming speed (U_{crit}) and total length (TL) for wild-caught and laboratory-reared red drum larvae over a common size range. The solid line is the regression for wild larvae and the broken line is for reared larvae. Regression lines were obtained using Tobit regression models. White triangles represent laboratory-reared larvae and black circles represent wild-caught larvae. Symbols above the horizontal solid line represent individuals that could not be fatigued, i.e. $U_{crit} > 22.2$ cm s^{-1}.

Tobit regression showed significant relationships between logU_{crit} and logTL for wild cohorts 2, 3 and 5, but no significant relationship for cohorts 1 and 4. Comparisons of the 95 % CI showed no significant differences in the slopes or the intercepts of the models (Table 2).

Regarding reared larvae, Tobit regression analyses showed that the slopes differed between some of the batches (Table 2). This is a result of the different size ranges: batches that included only relatively big larvae had smaller slopes than batches that included smaller larvae. This suggests a non-linear relationship between logU_{crit} and logTL when considering a wide range of larval size or ontogenetic state.

Table 2. (A) Results of the Tobit Regression Model, performed on censored data, test the relationship between logU_{crit} and logTL of wild-caught red drum larvae (all collections combined and each separate cohort) and red drum reared larvae (all larvae and batch 2, because this was the only batch with censored data); (B) Results of the Least Squares Model performed on non-censored data, test the relationship between logU_{crit} and logTL of each separate batch of reared larvae (batch 1, 3, 4 5 and 6). a = slope; b = intercept.

	$\log_{10}U_{crit} = a*\log_{10}TL + b$							
A)	**Tobit**	**a**	**95% CI**	**b**	**95% CI**	**n**	**X^2**	**P**
	Wild	**0.82**	**0.55 / 1.08**	**0.35**	**0.07 / 0.64**	**110**	**34.03**	**< 0.001**
	Cohort 1	2.65	-4.70 / 10.01	1.83	-10.62 / 6.96	6	1.07	0.301
	Cohort 2	1.60	0.82 / 2.37	-0.52	-1.37 / 0.33	30	15.02	< 0.001
	Cohort 3	0.89	0.36 / 1.42	0.33	-0.23 / 0.87	25	11.88	< 0.001
	Cohort 4	0.50	-0.07 / 1.07	0.68	0.06 / 1.29	30	2.99	0.084
	Cohort 5	1.03	0.09 / 1.97	0.15	-0.81 / 1.11	19	4.64	0.031
	Reared	**0.80**	**0.62 / 0.98**	**0.29**	**0.11 / 0.48**	**152**	**65.69**	**< 0.001**
	Batch 2	0.90	0.69 / 1.10	0.16	-0.05 / 0.36	96		
B)	**Least squares**						**R^2**	**P**
	Batch 1	0.80	-0.18 / 1.77	0.32	-0.75 / 1.39	26	0.107	0.104
	Batch 3	1.93	1.79 / 2.07	-0.75	-0.86 / -0.63	115	0.872	< 0.001
	Batch 4	1.13	0.79 / 1.47	-0.07	-0.39 / 0.24	36	0.570	< 0.001
	Batch 5	2.00	1.59 / 2.41	-0.69	-0.95 / -0.43	40	0.717	< 0.001
	Batch 6	1.86	1.32 / 2.41	-0.66	-1.02 / -0.31	29	0.646	< 0.001

Data for reared larvae covered a broader range of sizes and showed a clear picture of the ontogenetic change in U_{crit}. The parameter estimates for the piecewise regression were a = -0.83, b = 2.14, c = -1.36 (95% confidence interval = 1.61 – -1.10) and d = 0.84 (95% confidence interval = 0.80 – 0.87). Thus, the piecewise regression confirms the existence of a breakpoint in the relationship at logTL = 0.84. This breakpoint corresponds to 6.9 mm TL (6.2 - 7.5 mm TL) (Fig.4). To determine whether the slope of the upper branch of the piecewise regression would have been higher if considering the larvae that never fatigued, a tobit regression model was applied for fish larger than the breakpoint (6.9 mm TL), including the 9 larvae that did not fatigue. The resulting slope was 0.89 (95% CI: 0.74 - 1.04), which was not significantly different from the slope estimated by the piecewise model (0.78).

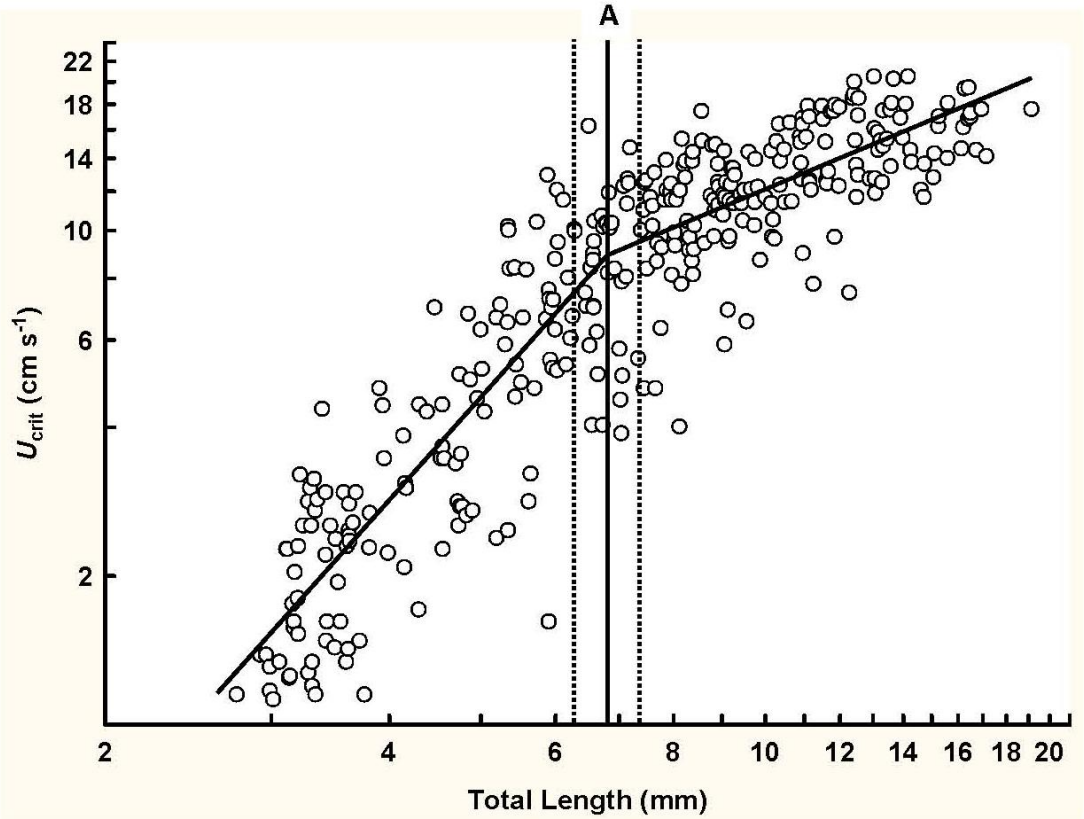

Fig 4. Ontogeny of critical swimming speed for reared red drum larvae. The solid line is a piecewise regression fitted to the data. Line *A* represents the breakpoint estimated by the piecewise regression (6.9 mm TL), and the dashed lines represent the 95% confidence interval

for the breakpoint. Piecewise regression equation is U_{crit} = -0.83 + 2.14 · logTL - 1.36 · (logTL – 0.84) · (logTL > 0.84).

The growth profile for larvae smaller than the breakpoint (6.9 mm TL) showed a clear U-shaped pattern, meaning that the snout and caudal fin grew faster (relative to eye diameter) than the central body sections. Growth profiles for larvae larger than the breakpoint lacked the U-shaped pattern (Fig.5).

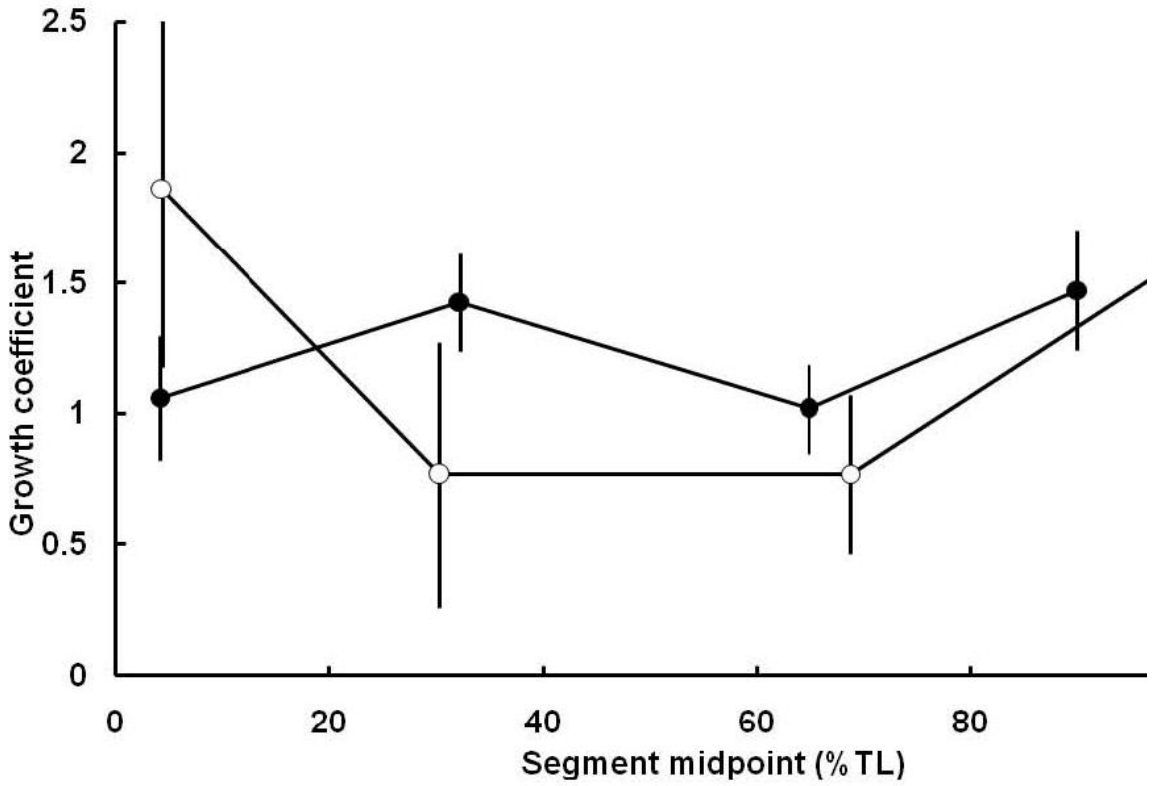

Fig 5. Growth profile for longitudinally measured body segments of red drum larvae (as illustrated in Fig 1). White circles represent pre-settlement larvae and black circles represent post-settlement larvae.

The transition to inertial environment, in red drum, is considered to take place at a Reynold's number (Re) > 1300 (Sarkisian 2005). Pre-settlement individuals, smaller than

7 mm TL, were clearly swimming in a viscous hydrodynamic environment (Re < 600), while post-settlement individuals, larger than 11 mm TL, swam in an inertial hydrodynamic environment.

DISCUSSION

In red drum larvae, critical swimming speed of wild and reared larvae increases with ontogeny (size) and there is substantial variation at any given size, as found in other species (Fisher et al. 2000, 2005; Clark et al. 2005, Leis et al. 2007). Size was a better predictor of U_{crit} than age when age was known (reared larvae). This has already been noticed in recent studies on the ontogeny of larval fish behaviour (e.g. Clark et al. 2005, Leis et al. 2007) and it is not surprising since "a given ontogenetic state is usually reached at a uniform size for a species, regardless of how long it takes to achieve it" (Fuiman & Higgs 1997).

The ontogenetic increase in critical swimming speed occurs in two phases, and this sharp change in the trajectory of swimming performance coincides with important changes in ecology, morphology, and hydrodynamics. There is an early phase of more rapid improvement which changes to a later phase of slower improvement when larvae attain 6.9 mm TL, which is approximately the size at which red drum leave the water column and settle into seagrass beds (Rooker et al. 1998). Thus, swimming ability increases more rapidly during the pelagic (pre-settlement) stage than the post-settlement stage. The period when larvae are less than 6.9 mm TL is also characterized by radical changes in body shape and structure. Specifically, growth profiles showed that the head and caudal regions grow faster than the other parts of the body at these smaller sizes. Finally, the critical swimming speed and size of larvae at the breakpoint between the two phases yield

a Reynolds number of about 650, which is the upper limit of the viscous hydrodynamic regime (Sarkisian 2005).

Previous studies have matched changes in swimming performance to developmental or ecological transitions, but the pattern of changes is not consistent across species. For shorthorn sculpin (*Myoxocephalus scorpius*), Guan et al. (2008) found that critical swimming speed improved steadily through pelagic stages until metamorphosis, after which improvements became slower. These authors suggested that this decrease was due to a habitat shift associated with settlement. In contrast, Fisher et al. (2000) found that the swimming ability of coral reef fish larvae increased more slowly in the early life stages and faster in later stages. Clark et al. (2005) found that until notochord flexion was complete, both speed and endurance of four species of temperate marine fishes (Sciaenidae, Sparidae, Percichthyidae) were limited and swimming performance improved markedly thereafter.

Accentuated growth of the head and tail during the larval period is a characteristic of many species (Fuiman 1983, van Snik et al. 1997, Gisbert 1999). These researchers have surmised that some of these morphological changes might have important locomotor consequences. Our assessment of relative growth and critical swimming speed provide empirical support to his claim.

Hydrodynamic conditions, as defined by fish size and swimming speed, can have a major influence on the energetic cost of swimming (Muller and Videler 1996, Hunt von Herbing 2002). Small, slowly swimming larvae swim under conditions that are dominated by viscous forces when $Re < 600$ (Fuiman and Batty 1997, Sarkisian 2005) and move to inertia-dominated swimming when $Re > 1300$ (Sarkisian 2005). At the breakpoint

between the two phases of ontogenetic increase in critical swimming speed, red drum larvae attain a Reynolds number of 640. Under these conditions, they enter the intermediate hydrodynamic regime and begin to escape the energetic burden of the viscous hydrodynamic regime at the same time that highly allometric growth produces a body shape that is suited to efficient locomotion in an inertial hydrodynamic environment.

Temperate species are considered to be poorer swimmers than tropical species (Stobutzki & Bellwood 1997). This may be due, in part, to differences in water temperature. It is well known that temperature influences both the physiology of fish larvae and the physics of the hydrodynamic environment in which larvae are swimming (Fuiman & Batty 1997). Differences in performance may also be attributed to different methodologies. Some studies of swimming performance focus on routine (unforced) laboratory swimming (e.g. Fuiman et al. 1999, Fisher & Bellwood 2003), others on swimming endurance (e.g. Dudley et al. 2000, Fisher & Wilson 2004), some on swimming in the sea (Leis & Carson-Ewart 1997) and others on critical swimming speeds (Fisher 2005, Fisher et al. 2005). Moreover, studies on reef fishes have concentrated on wild late-stage larvae (Stobutzki & Bellwood 1997, Fisher & Wilson 2004, Fisher et al. 2005), while studies on non-reef species have used laboratory-reared larvae at earlier developmental stages (Blaxter 1986, Miller et al. 1988). Other than these, variation can result from taxonomic and morphological differences (Leis & Carson-Ewart 1997, Stobutzki 1998, Dudley et al. 2000). Most studies compare temperate clupeiform, gadiform, or pleuronectiform larvae (Blaxter 1986, Miller et al. 1988) with tropical perciform larvae (Fisher et al. 2000, Fisher et al. 2005, Leis & Fisher 2006). For this reason, comparisons of swimming speed among taxa should take into account phylogeny, methodology, and developmental state. It only

makes sense to compare our results to other studies of U_{crit} measured on perciform species within the same size range.

When comparing critical swimming speeds of settlement-stage red drum larvae (9.7 – 22.2 cm s^{-1}) with U_{crit} for settlement stage larvae of reef fishes (11.3 to 61.5 cm s^{-1}, Leis & Fisher 2006), we found that red drum swimming capabilities are within the range of reef fishes, although below the mean (36 cm s^{-1}). Dudley et al. (2000) also reported that when tropical and temperate species of similar taxonomic groups are compared, differences in swimming performance were small.

At the family level, we found only two other studies of behavioural ontogeny in a sciaenid, the mulloway (*Argyrosomus japonicus*) (Clark et al. 2005, Leis et al. 2006b). Maximum U_{crit} for this species was 16.6 cm s^{-1}, at 10 mm SL (Clark et al. 2005) and *in-situ* maximum speed was 8.4 cm s^{-1} at 11.4 mm SL (Leis et al. 2006b). Thus, the larvae observed *in situ* were swimming much slower than their maximum capability (U_{crit}), as expected (Fisher & Bellwood 2002). When comparing maximum swimming performance of these two sciaenids, we conclude that at the same length (no information on ontogenetic state of *A.japonicus* is available), *Sciaenops ocellatus* swam 34% faster than *A. japonicus* (22.2 cm s^{-1} vs 16.6 cm s^{-1}). Temperature may not explain these differences, since both species were tested at similar temperature range: *S. ocellatus* at 24 to 26 ºC and *A.japonicus* at 22 to 24 ºC. Thus, these comparisons of taxonomically and ecologically similar species suggest that the relationship between swimming performance and size varies among species.

What is the ecological importance of swimming abilities for estuarine species? Tidal currents can be very strong in estuarine inlets. Specifically, there are strong currents near Lydia Ann Channel where collections of red drum larvae were made, and the larvae are probably at a great risk of being swept away during ebb tide if they leave the shallow seagrass beds (Brown et al. 2005). During the sampling period, currents at the channel varied between 5 and 100 cm s^{-1}, with an average speed of 35 cm s^{-1}. Red drum larvae are unable to swim against this average speed, however, currents become slower as the water enters the shallow areas, and one would expect that larvae use their strong swimming abilities to respond rapidly to high current flows in order to remain in the settlement habitat. Moreover, swimming abilities may also be needed to migrate further into the estuary. Rooker et al. (1998) suggested that post-settlement migration occurs in red drum, contrary to the "settle and stay" hypothesis (Bell & Westoby 1986). Post-settlement transport can occur as a result of advection or behaviour (Jenkins et al. 1997, 1999). Brown et al. (2005) used a numerical circulation model coupled to a particle transport model to examine the role of physical transport in the spatial distribution of settled red drum larvae in the Aransas Pass region and found that physical processes can explain substantial retention of particles in the absence of behaviour in some areas, but not in others. The settlement sites where the model reproduces temporal patterns in larval settlement are located closest to the Gulf, while the sites where the model performs the poorest are located further from the inlet in regions with indirect connection with the Gulf.

A study in Australia also found that a particle transport model explained the larval settlement at a site closest to the entrance of the bay but not at the site furthest from the entrance (Jenkins et al. 1997). The authors suggested that differences in the performance

of the model may be attributed to the hydrodynamic model itself, or due to the increasing importance of larval behaviour at sites located further from the centre of the estuary/bay. Bradbury et al. (2003) hypothesized that larvae reach a critical size at which active swimming overrides advection as the dominant factor in determining spatial pattern. These authors performed multiple ichthyoplankton surveys in Placentia Bay, Newfoundland, and found that the changes in spatial distribution patterns during the egg and early larval period clearly indicated the importance of advection. However, the distribution of bigger larvae of the most abundant species in the area (cod, capelin and cunner) indicated these are increasingly capable of regulating their distributions (Bradbury et al. 2003), probably due to their strong swimming abilities (Williams et al. 1996).

It is frequently assumed that swimming performance of reared larvae is inferior to that of wild larvae (e.g. Blaxter 1975), although few direct comparisons have been made (e.g. Smith & Fuiman 2004). Using reared larvae may not provide realistic results if their performance in the laboratory is poorer than that of wild larvae. Our study demonstrated that reared and wild larvae did not differ in swimming performance (measured as U_{crit}), suggesting that U_{crit} measured in lab-reared fish may accurately reflect swimming abilities of wild red drum. However, this can only be concluded for post-settlement larvae, since we did not catch wild larvae smaller than 8.25 mm TL. Smith & Fuiman (2004) found significant differences between reared and wild red drum larvae in routine speed at some developmental stages, but not all. They did not measure critical swimming speed.

It is important to note that U_{crit} is a laboratory measure of forced performance and it is unlikely that larvae are able to sustain maximum critical speeds in the wild for extended

periods. An approach that could clarify the actual behaviour of red drum larvae in the wild would be to follow larvae *in situ*. Trnski (2002) and Leis et al. (2006a, b) have shown that it is possible to estimate swimming speeds of tropical fish larvae *in situ*. There was a strong correlation between U_{crit} and *in-situ* speed for three temperate species and 11 tropical species (Leis & Fisher 2006, Leis et al. 2006b) where *in-situ* speeds averaged 50% of U_{crit} (Leis & Fisher 2006). This relationship makes it possible to estimate swimming speeds of larvae in the ocean from an easy and convenient laboratory measure, such as U_{crit} (Leis & Fisher 2006). It would be useful to confirm this relationship for red drum, but it must be recognized that the waters occupied by red drum larvae are much more turbid than those in which *in-situ* measurements have been made and that it might not be possible to achieve the same success.

Additional studies on the behavior of early life histories of fishes, and in particular on swimming abilities, are essential to our knowledge. Only a more realistic view of swimming behavior will help us to understand and predict the dispersal and retention patterns of species in coastal zones.

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CHAPTER IV. THE INFLUENCE OF CONDITION ON SWIMMING BEHAVIOUR

Does condition of gilthead seabream (*Sparus aurata*, Linnaeus 1758) larvae influence swimming behaviour in the pre-settlement phase?

Faria, A; Chícharo, M. and Gonçalves, E. (*submitted*). Does condition of gilthead seabream (*Sparus aurata*, Linnaeus 1758) larvae influence swimming behaviour in the pre-settlement phase? *Submitted to Journal of Aquatic Biology*

ABSTRACT

Body condition in larval fishes is an important determinant of survival in the natural environment. However, few studies correlate condition with any behavioural trait critical for survival, such as swimming. In the present study we evaluated critical (U_{crit}), routine and endurance swimming abilities in pre-settlement larvae (22 d post-hatch) of gilthead seabream (*Sparus aurata*), reared in different feeding treatments: fed and unfed larvae of up to 3 d for U_{crit}, and up to 2 d for routine and endurance performances. The possible use condition indices (RNA/DNA ratio and Fulton's condition factor) as a proxy for swimming performance was also investigated. Feeding treatment (fed and unfed larvae) did not influence the relationship of speed (critical or routine) with length. However, in the endurance experiment, fed individuals swam twice as far as the unfed larvae (19.7 km for fed larvae and 9.5 km for unfed larvae). RNA/DNA ratio was higher in fed larvae in the U_{crit} experiment, but significant effects were only detectable after a 3 d period of starvation. Fulton's condition factor was significantly higher in fed larvae only in the endurance trial, which suggests that growth (in weight) of starved larvae was affected by long-term swimming. Taken together, these results suggest the possibility that foraging and orientation behaviours (activities in which critical and routine speeds might be involved) are not affected by a reduction of feeding for some days, but sub-lethal effects of starvation may affect dispersal potential (for which endurance swimming is critical), and therefore compromise subsequent recruitment to the adult population.

KEYWORDS: Gilthead seabream, swimming performance, nutritional condition, sub-lethal effects, RNA/DNA ratio, Fulton's condition factor.

INTRODUCTION

Early life stages of marine fishes typically experience high rates of mortality, with strong implications on future recruitment. Several concepts, such as Hjort's (1914) 'critical period' hypothesis, Cushing's (1975) 'match-mismatch' hypothesis, and Lasker's (1975) 'stable ocean hypothesis', have been used to link larval survival (and subsequent recruitment) to feeding success during larval stages. Additionally, predation is also considered a major cause of mortality in larval fishes (Bailey & Houde 1989). These two factors can act together to increase mortality rates, as feeding success influences both growth and development of larvae, thus increasing the likelihood of predation on smaller and weaker individuals. Larvae in better condition are often those able to grow faster and attain larger sizes, reducing the duration of the early life stage. Faster growing larvae will thus attain larger sizes more quickly (the 'bigger is better' concept, Miller et al. 1988), thereby escaping predation through a size refuge (the 'growth-rate' concept, Bailey & Houde 1989). A more recent hypothesis receiving considerable interest is the 'ocean triad' hypothesis (Bakun 1996), in which enrichment, concentration, and transport-and-retention processes are all considered key factors influencing recruitment variability in marine fish populations.

Several studies indicate that high larval condition can be correlated with increased growth rate and enhanced recruitment or juvenile survival under natural conditions (Searcy & Sponaugle 2001, Bergenius et al. 2002, McCormick & Hoey 2004, Sponaugle et al. 2006), but few attempts have been made to correlate larval condition with any behavioural function critical for larval survival, such as swimming behaviour (e.g. Laurence 1972, Yin & Blaxter 1987, Chick & Van den Avyle 2000). Swimming is one of the most important behaviours in larval fish, determining to a large extent the success of

predator avoidance, prey capture and dispersal potential (Reidy et al. 2000, Armsworth 2001, Plaut 2001, Fisher & Wilson 2004): larvae in good condition possess greater swimming abilities and responsiveness to predators than larvae in poor condition (Chick & Van den Avyle 2000, Grorud-Colvert & Sponaugle 2006).

Larval condition may be estimated by a variety of morphometric, biochemical, histological or otolith growth indices. Fulton's condition factor *k* is a morphometric index commonly used as indicator of an individual's general well-being and is based on the assumption that heavier fish for a given length are in better condition (Ricker 1975, Suthers 1998). The RNA/DNA ratio is a widely used biochemical index of nutritional condition and recent growth of larval fishes (e.g. Clemmesen et al. 1997, Chícharo 1998, Buckley et al. 1999, Caldarone et al. 2003). It is based on the assumption that the amount of DNA, the primary carrier of genetic information, is stable under changing environmental situations within the somatic cells of a species, whereas the amount of RNA directly involved in protein synthesis, varies with age, life-stage, organism size, disease-state and with changing environmental conditions (Bulow 1970).

In the present study we aim to examine the influence of starvation on condition (measured through the RNA/DNA ratio and Fulton's condition factor) and swimming behaviour of gilthead seabream larvae (*Sparus aurata*, Linnaeus 1758). Three of the most common measurements of swimming capability (routine speed, critical speed and swimming endurance) were measured on larvae that had either been fed *ad libitum* or starved for 1, 2 or 3d. Pre-settlement stages were chosen because this phase is often characterized by very high mortality rates (Carr & Hixon 1995, McCormick 1998, Almany & Webster 2006, Leis 2006), with obvious implications for the subsequent survival and recruitment to the

adult population (Searcy & Sponaugle 2001, Hoey & McCormick 2004, Grorud-Colvert & Sponaugle 2006). We also investigated whether condition indices could be used as a predictor of swimming performance, and tested the hypothesis that larvae in poor condition have reduced swimming performance, which, in turn may have a significant impact on survival.

MATERIAL AND METHODS

Larvae. *Sparus aurata* (Linnaeus 1758) larvae were obtained from the fish hatchery TIMAR (Algarve, Portugal), at 22 days post hatching (dph) and were maintained in 20 l aquaria filled with filtered seawater, with constant slight aeration and a photoperiod of 13L:11D. Larvae were raised under a semi-closed circuit of filtered natural seawater originating from the nearby coast. Salinity was kept constant at 37 PSU and temperature ranged from 20.6 to 22.6 ºC.

Larvae were randomly distributed in two rearing aquaria, one aquarium with larvae fed *ad libitum* (*Artemia* nauplii) and the other aquarium with larvae deprived of food for a minimum of 24 h before the experimental tests. Three behavioural tests were performed in order to evaluate the influence of nutritional condition on swimming performance: critical swimming, routine swimming and endurance swimming. For each experiment, fed and unfed larvae were tested (see Fig. 1). Critical speed (U_{crit}) of fed larvae was measured at 25, 30 and 35 dph, whereas unfed (24, 48 and 72 h of food deprivation) larvae were measured at 25, 30, 35, 36 and 37 dph. Routine speed of fed and unfed (24 and 48 h of food deprivation) larvae was measured at 25, 36 and 50 dph. Swimming endurance was measured on late stages (50 dph) of fed and unfed (48 h of food deprivation) larvae.

For each experimental case, 8 to 12 larvae were tested. After the experiment, larvae were put in liquid nitrogen and stored at – 80 ºC for subsequent RNA/DNA analysis. The larvae were freeze dried and weighted before the biochemical analysis. Notes on ontogenetic progress were taken under a stereomicroscope and standard length (SL) was measured to the nearest 0.01 mm, using Image J software (version 1.38).

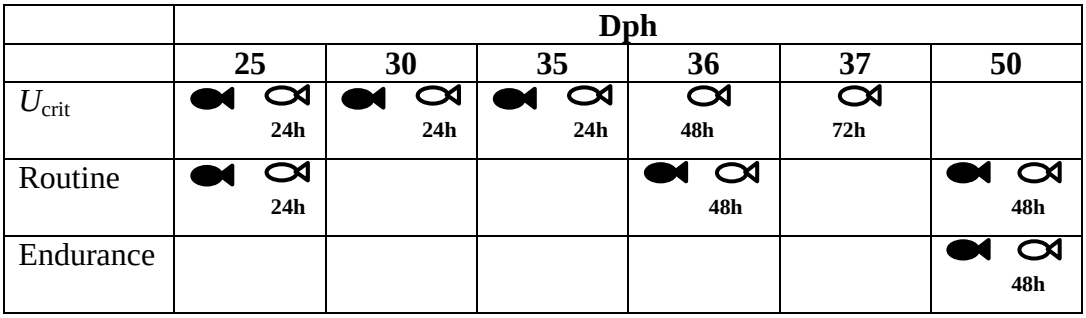

Fig 1. Behavioural tests conducted on *Sparus aurata* larvae of different ages. Black fish represent larvae fed *ad libitum*; white fish represent unfed larvae. The hours below white fish indicate the starvation period. U_{crit}: critical swimming speed; Rout: routine swimming speed; Endur: swimming endurance.

Critical swimming speed. U_{crit} was measured using a swimming chamber, following the protocols of Stobutzki & Bellwood (1994, 1997). The chamber was made of clear Perspex with 6 parallel swimming lanes, each 30 mm wide, 50 mm high and 180 mm long. A removable lid allowed introduction and removal of fish from the lanes. A strip of black tape on the top of the lid provided fish with a visual reference to maintain position in the flow, and a mesh screen was placed at the upstream and downstream ends of each lane to retain larvae. A section of flow straighteners, 40 mm long, was placed at the upstream end of each lane to minimize turbulence. Previous work demonstrated that at the typical U_{crit} water velocity was not significantly different between the centre of the lane and 5 mm from the wall (Stobutzki & Bellwood 1997, Stobutzki 1998, Fisher et al. 2000). Experimental observations also confirmed that larvae did not show a depth preference in

the chamber. The maximum flow speed was 22 cm s^{-1}. For details on the swimming chamber characteristics see Faria et al. (2009).

In the morning of each experimental day, 8 to 12 fish were tested. One hour after feeding, larvae were carefully removed from the rearing tank using a small container and placed individually in large petri dishes and left undisturbed for 1 h to allow recovery from handling (Fuiman & Ottey 1993). After this period, larvae were transferred to the swimming chamber, one in each lane, and allowed to acclimate for 5 min at a flow speed of 1 cm s^{-1}. If any behavioural symptoms of stress, such as lying on the bottom or clinging to the sides, were observed after this acclimation period, the individual was removed and replaced by another fish. Water temperatures in the chamber over the study period varied from 20.5 to 23 ºC. To measure U_{crit}, water velocity was increased by approximately 1.2 cm s^{-1} every 2 min until the larva was unable to swim against the current for 2 min. Calculation of U_{crit} followed Brett (1964):

$U_{crit} = U + (t/t_i \cdot U_i)$,

where U is the speed of the penultimate increment, U_i is the velocity increment, t is the time swum in the final velocity increment, and t_i is the time interval for each velocity increment (2 min). After the test, fish were immediately put in liquid nitrogen for condition analysis.

Routine swimming speed. The routine swimming test measured the mean speed of individual larvae over 120s during undisturbed activity in the absence of food. Eight similarly sized larvae were removed from the rearing tank the day prior to testing. Each of the eight larvae was then placed into a separate glass watch bowl (15 cm in diameter, 8 cm deep) in 1 l filtered sea water matching the temperature and salinity of their rearing

tank. The bowls had opaque, black sides. Before each experiment, the watch bowl was placed on a piece of white paper (to increase contrast on a video monitor) and under the video camera, and all but approximately 200 ml (1 cm depth) of water was carefully siphoned from the watch bowl to reduce measurement error caused by a possible depth change by the larva. The larva was left undisturbed for 2 min., and then its behaviour was recorded for two additional minutes. All eight larvae were tested two more times each, in sequence, for a total of three replicates per individual. After the trials, video footage was digitized at a frame rate of 1 s^{-1}, using the digital video software (Zoom Browser EX 5.7). After the test, fish were also immediately put in liquid nitrogen for condition analysis.

Swimming endurance. Swimming endurance was measured by swimming larvae continuously at a single speed until exhaustion. The speed of 12 cm s^{-1} (approximately 50% of the maximum U_{crit}) was chosen as a reference. During daylight hours, larvae were constantly observed and the exact time to fatigue was recorded. At night, larvae were observed every 6 h, and the time to fatigue was calculated as the midpoint between when the larva was last seen swimming and when it was found no longer swimming. The swimming duration was converted in distance swum (km) using the flow speed. The larvae do not actually swim this distance relative to any fixed point, but they do so relatively to the moving water in which they swim. The values are therefore given as km swum.

Condition indices. RNA and DNA were measured with the microplate fluorescent assay (MFA) of Wagner et al. (1998). The MFA assay is a modification of the sequential fluorometric method of Bentle et al. (1981), in which DNA and RNA in a single sample are determined sequentially by the addition of DNase and RNase, using Ethidium

bromide (EB) as fluorescent dye (see Caldarone et al. 2001 for details). Wagner et al. (1998) modified the sequential fluorometric method to the MFA with 96-well microtiter plates by adopting a sarcosyl extraction technique and eliminating the DNase step. The larvae were individually homogenized by sonication (3 pulses 50 A during 1 min) with cold sarcosyl extraction buffer. The volume of extraction buffer was 500 µl (0.5%). The samples then were shaken for 1 h at room temperature on a vortex mixer equipped with a multiple-vial head. Next, they were centrifuged (12.000 × g) for 15 min to separate insoluble larvae remains. The samples were subsequently diluted 1:10 with Tris buffer to reduce the sarcosyl concentration to 0.05%. In each run, duplicate 50 µl aliquots of supernatants of the samples and duplicates of 0, 0.6, 1.1, 1.7 and 2.3 µg ml^{-1} DNA standard solutions (λ-phagus 0.25 µg $µl^{-1}$ from Roche) and 16s–23s *E. coli* RNA (4 µg $µl^{-1}$), from Roche and 0, 3.6, 7.3, 10.9 and 14.6 µg ml^{-1} RNA standard solutions (16s–23s *E. coli* 4 µg $µl^{-1}$ from Roche) were transferred to 96-well microplates (type nuclon black round bottom). The average ratio of DNA and RNA slopes was 5.5 ± 0.8, which can be used to compare RNA/DNA ratio results determined by other protocols (Caldarone et al. 2006). EB solution (30µl) was added to each well, and the plates were shaken gently at room temperature for 15 min. The EB fluorescence was then scanned on a microplate reader (Biotek synergy HT model SIAFRTD) with 360 nm (excitation) and 590 nm (emission) (first scan- total fluorescence RNA and DNA). Following the first scan, RNase solution (30 µl, 0.12µg ml^{-1}) was added to each well and the concentration of DNA calculated directly by the standard curve. The concentration of RNA was determinate indirectly by subtraction of DNA fluorescence (second scan) from total fluorescence (first scan).

Fulton's condition factor was directly determined from morphometric data using the formula:

$K = 100W_t/L^3$,

where W_t = total wet weight (mg), and L = length (SL in mm) (Ricker 1975). For simplicity, it is hereafter referred to as Fulton's *K*.

Data analysis. The relationship between swimming performance (U_{crit} and routine speed) and standard length (SL) was examined by regressions of swimming speed against size. To determine the best predictor of performance, values of U_{crit} and routine swimming speed were regressed against SL using linear, power, logarithmic and exponential models. All models returned similar R^2; hence we report only linear relationships. Data on endurance were analysed by comparing the mean time swum for fed and unfed fish using a two sample *t*-test, after testing for normality assumptions. To determine whether the slopes of the regressions of swimming performance on size differed among the two feeding treatments, an analysis of covariance (ANCOVA) was performed with U_{crit}, routine speed and endurance as dependent variables, feeding treatment as the fixed factor, and size (SL) as the co-variate. Regression analysis and ANCOVA were also used to analyse the relationship between condition indices (RNA/DNA ratio and Fulton's *K*) and size (SL), and condition indices and swimming performance (U_{crit}, routine speed and endurance), for each feeding treatment. All statistical tests were conducted using STATISTICA software (Version 6.0).

RESULTS

A total of 142 larvae were tested in the swimming experiments, of which 81 belonged to the unfed group and 61 to the fed group. Larvae from both groups showed a significant

and constant growth in standard length (SL) up to the end of the experiments (Fig. 2). Overall, no significant effects of starvation on growth (length) were detected ($F_{(1,134)}$ = 3.87, p = 0.05). Standard length of the fed group varied between 6.2 and 15.7 mm and SL of the unfed group varied between 6.1 and 14.6 mm.

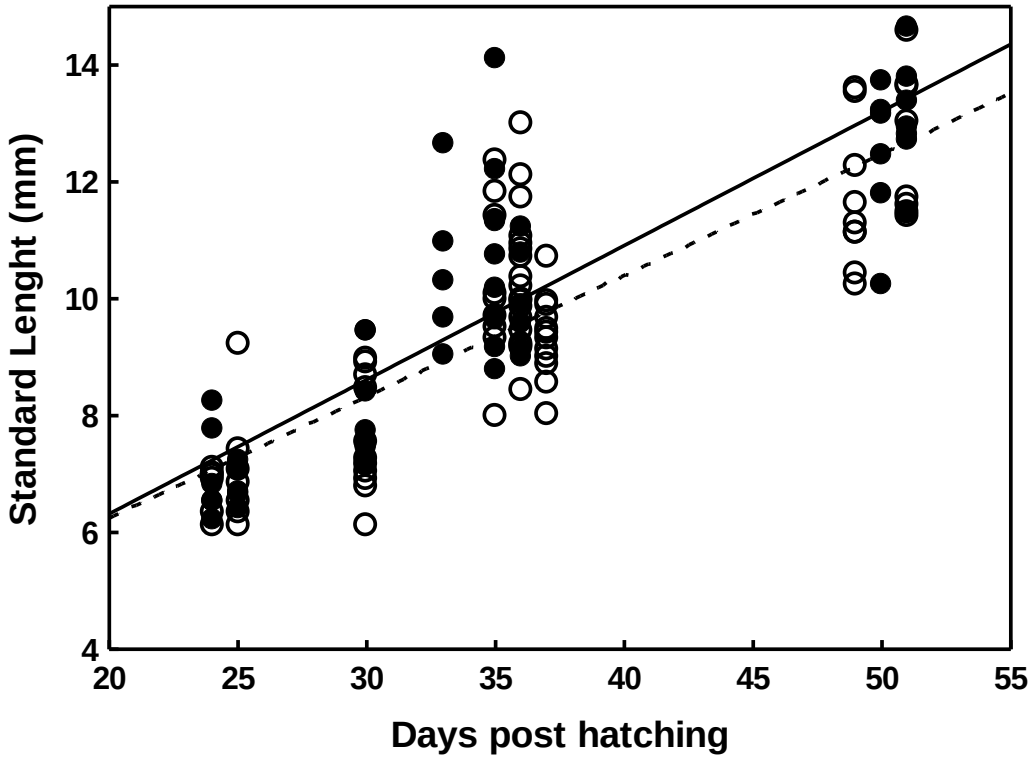

Fig 2. Changes in standard length (SL) with age (days post hatching) of fed (black circles) and unfed (open circles) larvae of *Sparus aurata*. The solid line is the regression line for fed larvae (y = 1.72 + 0.23x, R^2 = 0.75) and the broken line is the regression line for unfed larvae (y = 2.09 + 0.21x, R^2 = 0.68).

(1) Effect of feeding treatment on swimming performance of pre-settlement gilthead seabream larvae

A total of 48 larvae of the unfed group and 30 larvae of the fed group were tested for critical swimming trials. The U_{crit} values ranged from 3.0 to 19.3 cm s^{-1} or 4.5 to 20.9 body lengths (bl) s^{-1} for the group of fed larvae, over the size range of 6.2 to 14.1 mm (SL) and, as expected, swimming speeds increased with length ($F_{(1,28)}$ = 23.8, p < 0.0001)

(Table 1, Fig. 3). Age was also a good predictor of U_{crit}, explaining more of the variation in speed (64%) than size (46%) in fed larvae (Table 1). When analysing differences in swimming performance of unfed larvae tested in different periods of starvation (24, 48 and 72 h), ANCOVA revealed no differences in the U_{crit} and length relationship ($F_{(2,44)}$ = 0.07, p = 0.93), and therefore these larvae were treated as just one group (unfed group). Critical speed of the unfed group revealed a significant relationship with length ($F_{(1,46)}$ = 58.5, p < 0.0001) (Table 1, Fig. 3), ranging from 1.1 to 18.9 cm s^{-1} (1.5 to 20.2 bl s^{-1}) over the size range of 6.1 to 12.9 mm (SL). Variation among individuals in performance at any ontogenetic stage was large (Fig. 3). Surprisingly, there were no differences in slopes of the regressions of U_{crit} on size of fed and unfed larvae (ANCOVA, $F_{(1,75)}$ = 0.39, p = 0.53).

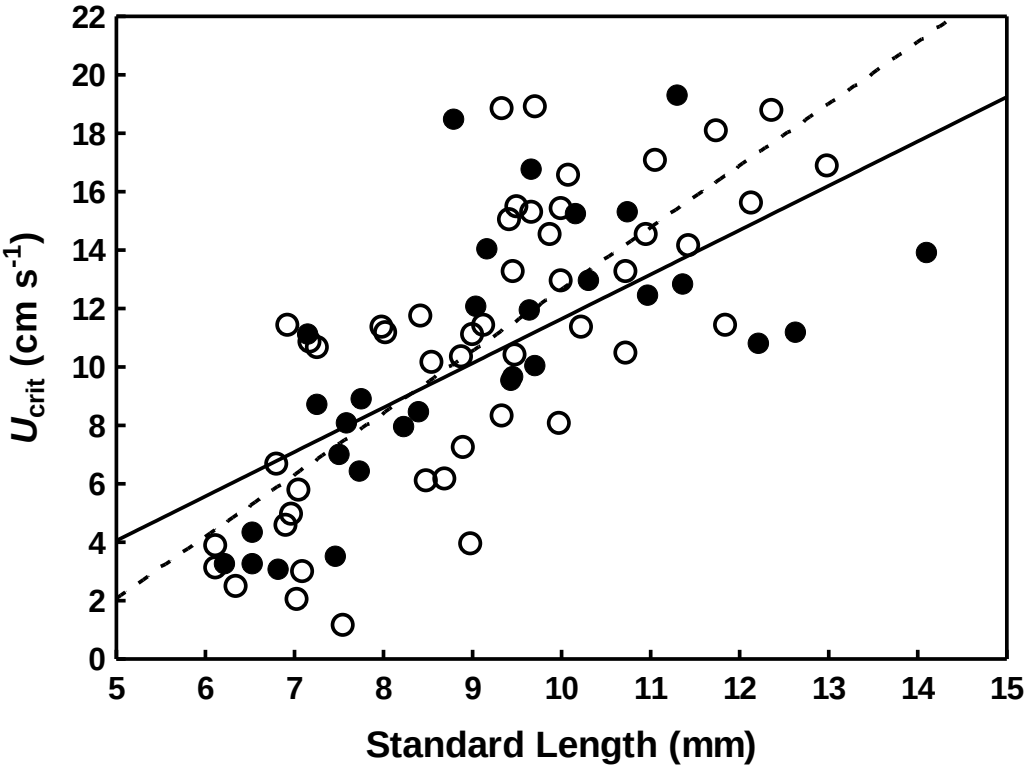

Fig 3. Ontogenetic trend for critical swimming speed (U_{crit}) of fed (black circles) and unfed (open circles) larvae of *Sparus aurata*. Each symbol represents results for an individual larva. The solid line is the regression line for fed larvae and the broken line is the regression line for unfed larvae. (See Table 1 for regression formulae).

Table 1. Relationships between critical speed (U_{crit}) and size (SL), U_{crit} and age (days post hatch - dph), routine speed and size (SL) and routine speed and age (dph) of fed and unfed groups. CI = confidence interval, ns = not significant ($p > 0.05$). Swimming speed is the dependent variable and size the independent variable in every relationship.

	Relationship	**R^2**	**p**	**Slope ± 95% CI**
U_{crit} (cm s^{-1}) *vs* size				
Fed	y = 1.52x – 3.54	0.46	<0.001	1.52±0.66
Unfed	y = 2.12x – 8.51	0.56	<0.001	2.12±0.56
U_{crit} (cm s^{-1}) *vs* age				
Fed	y = 0.87x – 16.65	0.64	<0.001	0.87±0.27
Unfed	y = 0.84x – 17.21	0.55	<0.001	0.84±0.22
Routine (mm s^{-1}) *vs* size				
Fed	y = 0.03x + 4.65	0.0005	ns	0.03±0.77
Unfed	y = -0.73x + 14.05	0.14	ns	-0.73±0.88
Routine (mm s^{-1}) *vs* age				
Fed	y = 0.01x + 4.50	0.001	ns	0.01±0.20
Unfed	y = -0.17x + 13.01	0.14	ns	-0.17±0.20

For the routine swimming experiments, a total of 23 unfed and 21 fed larvae were tested. The relationship between routine swimming speed and size was non-linear (fed: $F_{(1,19)} = 0.01$, $p = 0.92$; unfed: $F_{(1,21)} = 3.53$, $p = 0.07$), with highest speeds in larvae less than 11 mm and a bell-shaped pattern (Table 2, Fig.4). Routine swimming speed of the fed group ranged from 0.9 to 17.2 mm s^{-1} (0.08 to 1.75 bl s^{-1}) over the size range of 6.4 to 15.7 mm (SL) and, for the unfed group, from 0.8 to 15.1 mm s^{-1} (0.07 to 1.98 bl s^{-1}) over the size range of 6.1 to 14.6 mm (SL) (Fig. 4). In both treatments, age was as good as size in explaining the observed variation (Table 1). ANCOVA also revealed no significant differences in routine swimming between fed and unfed larvae ($F_{(1,41)} = 1.33$, $p = 0.25$).

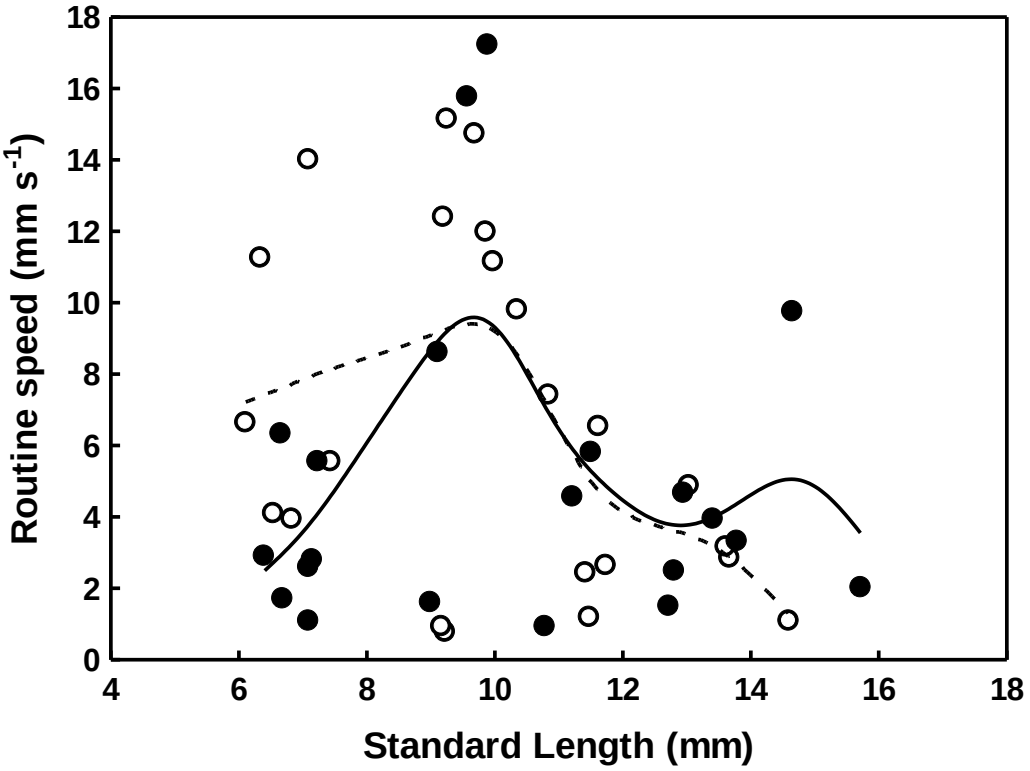

Fig 4. Ontogenetic trend for routine swimming speed of fed (black circles) and unfed (open circles) larvae of *Sparus aurata*. Each symbol represents results for an individual larva. The trend lines (thick line: fed larvae; broken line: unfed larvae) represent a distance-weighted least-squares smoothing function and are not meant to imply statistical significance; they provide a sensitive method for revealing non-salient overall patterns in the data. (See Table 1 for regression formulae).

Table 2. Relationships between condition indices (RNA/DNA ratio and Fulton's *K*) and size (SL in mm) of fed and unfed groups (data for all swimming tests pooled together). CI = confidence interval, ns = not significant ($p > 0.05$).

	Relationship	**R^2**	**p**	**Slope ± 95% CI**
RNA/DNA *vs* size				
Fed	y = 0.47x – 1.29	0.26	<0.001	0.47±0.22
Unfed	y = 0.38x – 1.47	0.22	<0.001	0.38±0.17
Fulton's *K vs* size				
Fed	y = 0.001x + 0.27	0.0003	ns	0.001±0.01
Unfed	y = -0.017x + 0,40	0.03	ns	-0.017±0.02

For the endurance experiment, a total of 24 fish (12 fed and 12 unfed) were swum at a constant speed of approximately 12 cm s^{-1}. Of these, two larvae in each treatment were lost during the experiment, thus results report swimming performance of 10 larvae for each group. The fed group presented significantly higher swimming duration than the unfed group ($t = -5.16$, $df = 18$, $p < 0.0001$), with fed larvae swimming about twice as far as unfed larvae. Fed fish swam an equivalent mean distance of 19.7 km (range = 10.3-23.9), whereas unfed fish swam an equivalent mean distance of 9.5 km (range = 4.4-14.1) (Fig. 5).

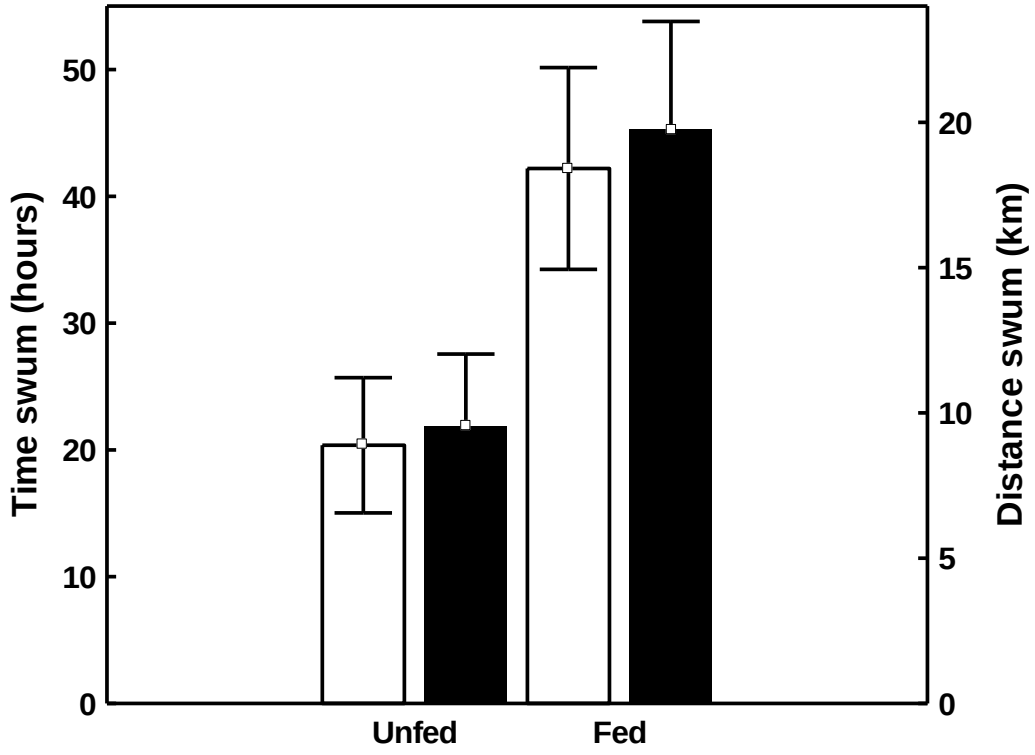

Fig 5. Comparison of mean endurance swimming time (hours – white bars) and equivalent distance travelled (km – black bars) between fed and unfed larvae of *Sparus aurata*. Whiskers represent 95% confidence intervals (see text for statistical results).

(2) Effect of feeding treatment on condition of pre-settlement gilthead seabream larvae and relationship between condition and swimming performance

The condition indices (RNA/DNA ratio and Fulton's *K*) were not correlated with each other, and only RNA/DNA ratio had a significant and positive relationship with length in both feeding treatments (Table 2). An overall significant difference in the relationship of RNA/DNA ratio with length was found between fed and unfed groups when analyzing all data pooled together (without discriminating the swimming test performed) ($F_{(1,134)}$ = 10.80, p = 0.001; Fig. 6A). However, the relationship between RNA/DNA ratio and length was significantly different between treatments only for U_{crit} ($F_{(1,75)}$ = 22.47, p < 0.0001). For routine and endurance trials, no significant differences were found (routine: $F_{(1,41)}$ = 1.06, p = 0.31; endurance: $F_{(1,12)}$ = 0.007, p = 0.93). On the contrary, the relationship between Fulton's *K* and length was not significantly different between feeding treatments when analysing all data together ($F_{(1,134)}$ = 3.49, p = 0.06; Fig. 6B), but a significant difference was found for the endurance test ($F_{(1,12)}$ = 7.81, p = 0.02) with fed larvae presenting higher Fulton's K indices (fed: average = 3.7, range = 2.7–4.6, N = 6; unfed: average = 2.5, range = 1.2–3.8, N = 9) .

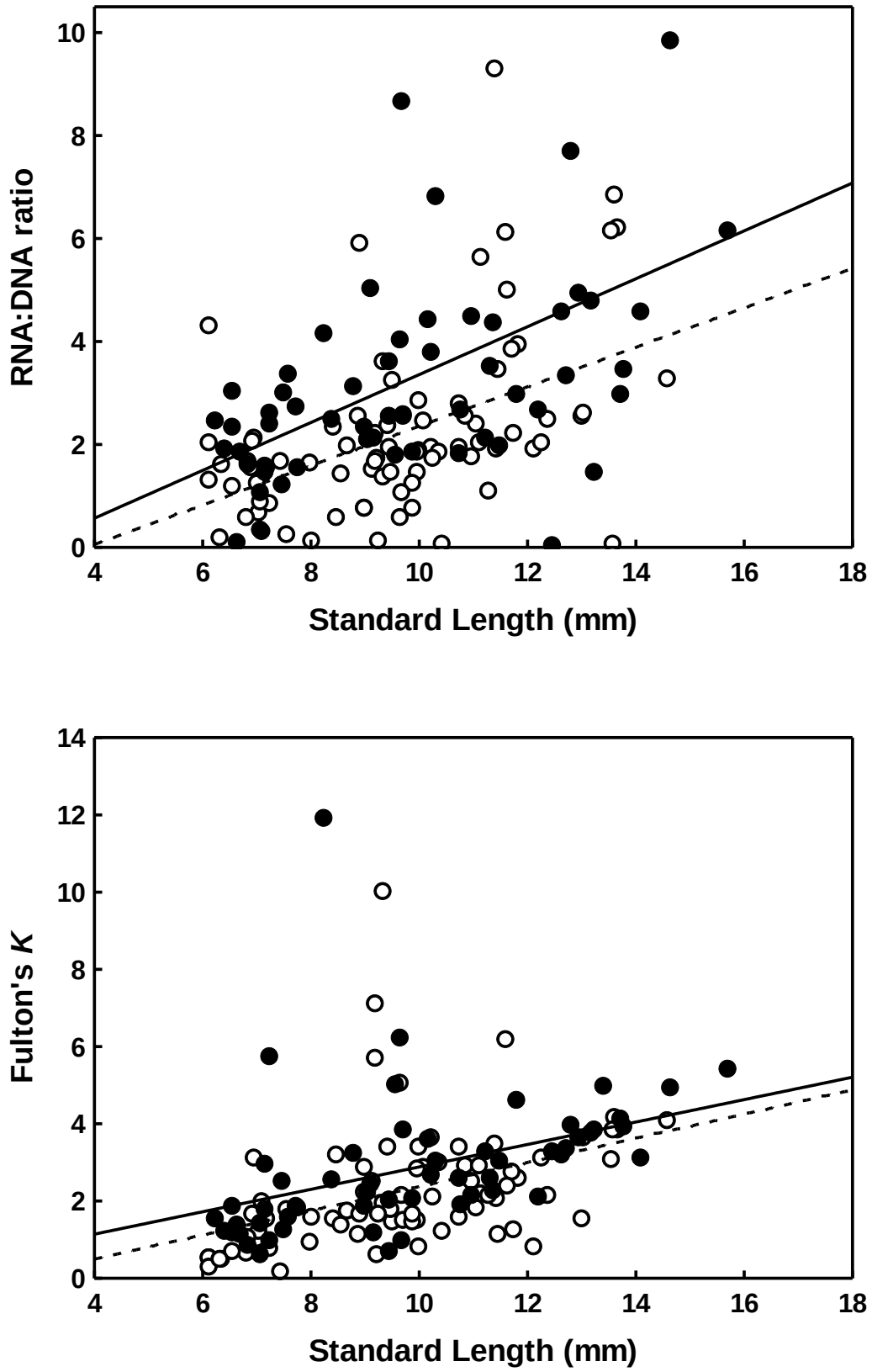

Fig 6. Relationship between standard length (SL) and the condition indices of fed (black circles) and unfed (open circles) larvae of *Sparus aurata*. Each symbol represents results for an individual

larva. The solid line is the regression line for fed larvae and the broken line is the regression line for unfed larvae. Top – RNA/DNA ratio; Bottom – Fulton's *K*. (See Table 2 for regression formulae).

The relationship between RNA/DNA ratio and swimming performance varied according to the swimming test. RNA/DNA ratio *vs*. U_{crit} proved to be different between the two feeding treatments ($F_{(1,75)}$ = 24.02, p < 0.0001). The fed group had a positive and significant relationship between nutritional condition and critical speed ($F_{(1,28)}$ = 7.13, p = 0.01), while for the unfed group this relationship was marginally non-significant ($F_{(1,46)}$ = 3.56, p = 0.07). In contrast, the relationship RNA/DNA ratio *vs*. routine speed showed no differences between feeding groups ($F_{(1,41)}$ = 0.53, p = 0.47), and a significant but negative relationship between RNA/DNA ratio and routine speed for the unfed group was registered ($F_{(1,21)}$ = 7.38, p = 0.01). Finally, for the endurance test, there was no effect of feeding treatment on the relationship RNA/DNA ratio *vs*. distance swum ($F_{(1,12)}$ = 0.003, p = 0.96), and neither regressions of condition on performance were significant (unfed: $F_{(1,7)}$ = 0.0007, p = 0.98; fed: $F_{(1,4)}$ = 0.07, p = 0.81). Additionally, the relationship of Fulton's *K* with swimming performance was not affected by feeding treatment in any swimming measure (U_{crit}: $F_{(1,75)}$ = 1.97, p = 0.16; routine: $F_{(1,41)}$ = 0.52, p = 0.47; endurance: $F_{(1,12)}$ = 0.78, p = 1.39), and regressions of condition on performance were also not significant (Table 2). For each experimental test, fed groups had higher Fulton's *K*, RNA/DNA ratios, RNA and DNA concentrations (µg nucleic acid/mg dry weight of larvae) compared to unfed larvae (see Table 3).

Table 3. Nucleic acid content and Fulton's *K* factor of analysed larvae for the fed and unfed groups in the U_{crit}, routine and endurance experiments (average ± standard deviation). N = number of larvae, SL = standard length, W = weight, DW = dry weight.

	N	SL (mm)	W (mg)	µg RNA/larva	µg RNA/mg DW	µg DNA/larva	µg DNA/mg DW	RNA/DNA	Fulton's *K*
U_{crit}									
Unfed	48	9.07±1.75	1.82±1.50	134.95±188.50	82.55±95.39	75.03±89.30	49.93±46.96	1.89±1.10	0.22±0.16
Fed	30	9.13±1.98	2.31±1.97	471.39±934.48	160.78±175.17	122.03±209.93	46.97±47.12	3.31±1.59	0.28±0.24
Routine									
Unfed	23	9.96±2.48	3.28±2.87	196.67±195.86	117.88±143.29	104.18±119.76	93.83±118.64	2.73±2.31	0.25±0.18
Fed	21	10.26±2.97	4.09±3.64	492.22±454.55	215.67±285.12	134.78±72.84	112.08±186.43	3.56±3.17	0.29±0.15
Endurance									
Unfed	9	11.70±1.22	3.67±1.82	248.13±171.81	74.54±44.78	383.04±605.97	142.89±281.14	2.64±2.34	0.21±0.05
Fed	6	12.44±1.28	5.88±1.74	717.93±670.43	114.28±89.87	335.16±416.92	52.22±59.28	2.66±1.69	0.30±0.05

DISCUSSION

As found in other studies (Clark et al. 2005, Leis et al. 2006a, b, 2007, 2009a, b, Faria et al. 2009), *Sparus aurata* larvae became more competent swimmers with growth. Critical swimming speed (U_{crit}) increased throughout development, for both fed and unfed larvae. Nevertheless, variation at any size was large, and size itself only explained 46% and 56% of the variation in critical swimming performance for fed and unfed groups, respectively. This result has been documented in other experimental works (Fisher et al. 2000, Clark et al. 2005, Leis et al. 2006a, b, 2007, 2009a, b, Faria et al. 2009) and suggests that other factors in addition to size are important in influencing swimming speed, such as larval condition (Leis & McCormick 2002).

Critical swimming speed recorded for gilthead seabream larvae in the current study ranged from 3.0 to 19.3 cm s^{-1} (14.5 to 20.9 body lengths per second - bl s^{-1}) for fed larvae and 1.7 to 18.9 cm s^{-1} (2.2 to 20.2 bl s^{-1}) for unfed larvae. Statistically, there were no differences between fed and unfed treatments in either slope or intercept, suggesting that a period of up to 3 d of food deprivation did not affect critical swimming performance in the pre-settlement stages of *S. aurata* larvae. Several studies have analysed critical speed of temperate Sparidae larvae. In particular, a recent work by Koumoundouros et al. (2009) have, as well, examined the ontogeny of critical swimming speed of pre-metamorphic (13.7-18.7 mm total length, TL) and post-metamorphic (20.4-34.3 mm TL) *S. aurata* larvae at different temperatures (15, 20, 25 and 28 °C). At 20 ºC, a temperature similar to the one used in the present study, and at early metamorphosis, average relative U_{crit} of larvae was 6.2 bl s^{-1} (Koumoundouros et al. 2009), which is notably lower than average relative speeds reported in our study (10.5 bl s^{-1}). Additionally, our larvae are on average much smaller (4.8-15.7 mm SL) than the ones

used in the Koumoundouros et al. (2009) study. These differences may be attributed to the experimental protocol itself, as the swimming chambers differed between studies, as did the speed increments and time intervals between each increment used in both cases. Although Hogan et al. (2007) concluded that U_{crit} is relatively robust to variations in methodologies (in what respects to length of the time interval), it seems fundamental to standardize procedures for these experiments, in order to allow meaningful comparisons, regardless of methodologies. The experimental set-up used in the present study followed the set-up first proposed by Stobutzki & Bellwood (1994, 1997), which has been adopted ever since for these type of protocols.

Recently, Pattrick & Strydom (2009) examined U_{crit} and endurance abilities of late-stage wild larvae of two other temperate Sparidae, *Diplodus capensis* and *Sarpa salpa*, using an experimental set-up similar to ours, and reported maximum U_{crit} values of 35 and 33 cm s^{-1}, respectively. These speeds are greater than the ones recorded in the present study for *Sparus aurata*. However, the larvae in Pattrick & Strydom (2009) study (8.9-21.3 mm) were larger than larvae in the present study (4.8-15.7 mm SL), which could account for these differences in maximum U_{crit} speed. Clark et al. (2005) studied the ontogeny of U_{crit} of two warm-temperate Sparidae, *Acanthopagrus australis* and *Pagrus auratus*, over a size range very close to the size range used in the present work, and U_{crit} speeds varied between 2 and 27 cm s^{-1}, which falls within the U_{crit} range for *Sparus aurata* here described.

Routine speed – the mean rate of travel during undisturbed activity (Fuiman et al. 1999) – is a measure of undisturbed swimming in the laboratory, often measured in small containers, and is not directly comparable to critical speed. Average routine speeds of

larvae in the experiments were low, ranging between 0.9 to 17.2 mm s^{-1} for fed larvae and 0.8 to 15.1 mm s^{-1} for unfed larvae, which represents *ca*. 4 to 8 % of their U_{crit}, respectively. As seen for U_{crit}, routine swimming speed did not differ between fed and unfed treatments, which again suggest that the starvation period used (2 d) is not enough to induce changes in the undisturbed behaviour of *S. aurata* larvae. As opposite to other studies on routine speed (e.g. Fuiman et al. 1999, Smith et al. 2004), in our study no significant relationship between routine swimming speeds and length or age was observed. However, the general trend showed a bell-shaped curve, with speed increasing to a certain size, after which it started to decrease. This reduced swimming activity after a certain size (10 mm SL, in the present study) might be related to behavioural changes associated with a benthic lifestyle, since these were pre-metamorphosing larvae. These changes in swimming performance and their relation to developmental or ecological transitions have been documented in several studies (e.g. Stobutzki & Bellwood 1994, Dudley et al. 2000, Guan et al. 2008), which report an improvement in swimming performance until metamorphosis, after which improvements became slower, or performance decreased. The reason for not observing a similar trend in U_{crit} might be related to the fact that in critical swimming experiments, larvae are forced to swim, as opposite to routine experiments.

The greatest impact of starvation on performance was seen on the endurance experiment: fed larvae swam twice as long as unfed larvae, with an average of 42.2 hours and 19.7 km, at 12 cm s^{-1}. The mean endurance results found are higher than values reported for settlement stage larvae of other Sparidae: in *Pagrus auratus*, 9.9 km, at a speed of 10 cm s^{-1} (Clark et al. 2005); *Sarpa salpa* and *Diplodus capensis*, 8 and 6 km, respectively, at a speed of 18 cm s^{-1} (Pattrick & Strydom 2009). These differences might be related to the

speed chosen for the endurance experiments, but can also be attributed to the ontogenetic stage itself. During swimming trials, lipids, carbohydrates and proteins are all used (Stobutzki 1997). Larvae that were fed *ad libitum* prior the experiment had clearly more energetic reserves than the ones that were starved for a 2 d period. These results suggest that reserves of unfed larvae were depleted faster than reserves of fed larvae. Therefore, the commonly used measurements of endurance swimming of larvae not fed during the trials seem more likely to provide an indication of the stored reserves of the larvae than a measure of how long (far) the larvae can swim in the field, except where ambient food concentrations are very low (Fisher & Bellwood 2001, Leis & Clark 2005). Fisher & Bellwood (2001) examined the effect of food on the sustained swimming ability of late-stage *Amphiprion melanopus* at 7 cm s^{-1} and reported an increased swimming distance from around 6.9 to 12.2 km when feeding larvae during the trial. Leis & Clark (2005) also found that swimming endurance of late stage larvae of six pomacentrid species was greatly increased by feeding, being able to swim at least twice as long as unfed larvae. Considering these results, one can speculate that endurance abilities of settlement stage larvae of *Sparus aurata* in the field, where larvae can stop to feed, will be greater than the ones found in this laboratory study.

The lack of food deprivation effects on short term swimming behaviours, such as U_{crit} and routine speed, has been reported in other studies. Laurence (1972) studied sustained swimming abilities and activity level of fed and starved largemouth bass (*Micropterus salmoides*) larvae at 19 ºC, and found that differences in swimming activity were only notable after a period of 4 d of starvation. Similarly, Yin & Blaxter (1987) reported decreased responsiveness and escape speed for starved herring, cod and flounder, reared at 9-10 ºC, but the effects of starvation were not evident until larvae had starved for

several days. Chick & Van den Avyle (2000) observed a similar pattern when examining the effects of feeding ration on the routine swimming speed of larval striped bass (*Morone saxatilis*). More recently, Skajaa & Brown (2007) concluded that escape responses in food-deprived cod larvae (*Gadus morhua*) were in general not affected by 3 d of food deprivation at 10 ºC. These studies support the evidence that critical and routine speeds are conserved and prioritized even in food deprivation scenarios. On the contrary, long-term performance, such as endurance swimming, is affected by short periods of starvation which suggests that, in terms of dispersal potential (for which endurance swimming is critical), starving larvae may not succeed.

Growing evidence suggests that body condition, measured physiologically as lipids, proteins, carbohydrates and standardized weight, may play a role in larval survival (Booth & Hixon 1999, Searcy & Sponaugle 2001, Booth & Beretta 2004, Hoey & McCormick 2004, Grorud-Colvert & Sponaugle 2006, Holmes & McCormick 2009). However, few studies have examined behaviour, and in particular swimming performance, as a function of larval condition. Although its validity has been questioned (for assuming isometric growth), Fulton's *K* has been widely used as a measure of body condition in fish larvae and recently settled recruits (e.g. McComirck & Molony 1993, Booth & Hixon 1999, Hoey & McCormick 2004, Grorud-Colvert & Sponaugle 2006, Holmes & McCormick 2009). Grorud-Colvert & Sponaugle (2006) studied the influence of condition on behaviour and survival potential of newly settled bluehead wrasse *Thalassoma bifasciatum* and reported that recruits fed for one week grew faster, had a greater Fulton's condition factor, swam faster and avoided simulated predators at faster speeds than recruits starved for the same period. In the present work, the relationship of Fulton's *K* and length in the critical and routine trials was not affected by feeding treatment, and this

is probably due to the fact that those swimming types are short term measures of performance, and weight itself is not affected during the trial. On the contrary, fed larvae tested in the endurance trial had a higher Fulton's *K* when compared to starved larvae, which may indicate that unfed larvae grew slower (in terms of weight) or lost more body mass.

RNA/DNA ratio has been shown to respond to changes in feeding conditions and growth in periods as short as one to three days in a variety of fish species and is a reliable growth rate estimator, which has been applied in numerous field assessments (Rooker & Holt 1996, Buckley et al. 1999, Gwak & Tanaka 2001, Chícharo et al. 2003). In the present study, the relationship of RNA/DNA ratio with size confirmed differences in nutritional status between the two feeding groups, but only on critical swimming experiments. This can be explained by the fact that only individuals tested in U_{crit} trials were starved for 3 d, as opposed to individuals tested on routine and endurance trials, which were starved for a 2 d period. These results seem to indicate that 3 d is the minimum starvation period necessary to induce changes in RNA/DNA ratio. In fact, latency has been demonstrated to be as short as 1-3 d in some fish species. For instance, Clemmesen (1994) reported that fed and starved herring (*Clupea harengus*) larvae (> 10 days) could be distinguished after 3 to 4 d using the RNA/DNA ratio. Similarly, Rooker & Holt (1996) reported that fed and starved larval and juvenile red drum (*Sciaenops ocellatus*) could be discriminated within 1 to 2 d of food deprivation using the same ratio. The relationship of RNA/DNA ratio with swimming performance only returned significant differences between feeding groups when analysing U_{crit} data. Since individuals tested in routine and endurance experiments were starved for 2 d, one can speculate that, in these trials, the relationship

between RNA/DNA and swimming performance could change if a longer starvation period was used, due to direct re-absorption of ribosssomes (Clemmesen 1994).

The RNA/DNA ratio in fed larvae correlated positively with critical speed, meaning that larvae with higher condition are capable of attaining higher critical speeds. Since the feeding treatment did not influence the critical swimming performance itself, but showed a significant effect on the RNA/DNA ratio, one can speculate that although unfed larva have a poorer nutritional condition, individuals still have the capacity of performing increased swimming speeds. Sjakaa & Brown (2007) also found that, despite the reduction in condition, measured as RNA/DNA ratio, escape responses of cod larvae were, in general, not affected by 3 d of food deprivation at 10 ºC. Interestingly, routine speed of unfed larvae showed a significant negative correlation with RNA/DNA ratio, suggesting that starving larvae spend more time swimming, which could be an indication of an increase in search activity looking for prey.

In spite of this, care should be taken when extrapolating the attributes of laboratory-reared larvae to ocean-caught larvae (Ferron & Leggett 1994). Larvae reared in the laboratory are usually fatter, exhibit less shrinkage, have less histological variation, less RNA and DNA relative to length (Ferron & Leggett 1994), and may require prey concentrations 2-3 orders of magnitude greater than in the field to survive (Suthers 1998). RNA/DNA ratio of fed larvae in the present study ranged from 2.66 to 3.56, and for unfed larvae, it ranged from 1.89 to 2.64. The critical value of RNA/DNA ratio for survival is species specific. To our knowledge, there are presently no reference studies on RNA/DNA ratio of wild-caught *S. aurata* larvae, or any other sparid which could be used for comparison. Nevertheless, unpublished data on nucleic acid content of wild-caught *Diplodus* sp. larvae

(M.A. Chícharo) seem to be comparable to the values reported in the present study for fed larvae. *Diplodus* sp., with a mean size of 11.7 mm (SL) ± 0.68 (SD) had a mean RNA/DNA ratio of 3.8 ± 1.04 (SD). Percentage of survival of unfed larvae or larvae reaching the point of no return (PNR) was not assessed in the present study, and thus we cannot define a critical value for the RNA/DNA ratio. However, given that our starvation experiments had repercussions on this biochemical index, we can speculate that field-caught larvae of *Sparus aurata* with a comparable RNA/DNA ratio to the one found in the present study for fed larvae will be in good condition. The lack of correlation between the two condition indices measured in this study supports the evidence that no single measure gives an adequate description of a fish's survival potential. Each measure will address specific questions regarding the fish's condition (McCormick & Molony 1993). Poor relationships between measures of condition have been reported in several studies (e.g. Kerrigan 1996, McCormick & Molony 1993, Hoey & McCormick 2004, Holmes & McCormick 2009). Latency (the temporal response of an index to a starvation period) is probably the reason why condition indices derived from the same individual are poorly correlated (Suthers 1998), and why most indices are unable to distinguish between increasing or decreasing condition.

Overall, our results seem to indicate that the impacts of nutritional condition on performance of pre-metamorphic larvae of *S. aurata* vary according to the swimming type being measured. A two to three day period of food deprivation did not affect routine and critical swimming behaviours, but both are short term measures of performance. However, endurance abilities were significantly affected by starvation. This suggests that even in poor nutritional condition, larvae may be able of performing escaping and foraging behaviours (activities in which critical and routine speeds might be involved),

but sub-lethal effects of starvation may affect dispersal potential by greatly reducing endurance swimming, and therefore compromise subsequent survival and recruitment to the adult population. Whether the condition indices measured in the present study can be used as a proxy of swimming performance of pre-metamorphosing *S. aurata* larvae remains to be proved, and for that matter, future studies need to define critical values of condition for survival and measure swimming performance on wild-caught larvae. Nevertheless, the simultaneous use of different types of swimming tests with body condition indices is a valuable tool for assessing the impact of starvation on larval behaviour and survival.

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Influence of starvation on the critical swimming behaviour of the Senegalese sole *Solea senegalensis* and its relationship with RNA/DNA ratios during ontogeny

Faria, A; Muha, T; Morote, E; Chícharo, M (*in press*). Influence of starvation on the critical swimming behaviour of the Senegalese sole *Solea senegalensis* and its relationship with RNA/DNA ratios during ontogeny. *Scientia Marina*.

SUMMARY

Food availability can affect larval survival directly through starvation and indirectly through the effects on larval growth rate, swimming performance and vulnerability to predators. In the present study we evaluate the effects of starvation on growth, nutritional condition and swimming behaviour of Senegalese sole *Solea senegalensis* throughout ontogeny (8 to 14 days after hatching). Biochemical analysis (RNA/DNA ratios) and behavioural experiments (critical swimming speed, U_{crit}) were conducted on larvae reared under 3 feeding treatments: fed (*ad libitum*), short-term starvation (starved for 48 h) and long-term starvation (starved for 96 h). Growth was significantly affected by feeding treatment, while only slight decreases on RNA/DNA ratio and swimming performance were registered. Late stage larvae of the three feeding treatments had slower critical speeds when compared to pre-flexion and flexion stages, which is probably related to the benthic life style acquired by the species in the end of the larval period. These physiological and behavioural changes are in accordance with previous results, which show that flatfish larvae are more resistant to starvation when compared to pelagic species and they become less active later in development.

KEYWORDS: Senegalese sole, *Solea senegalensis*, feeding treatment, starvation, RNA/DNA ratio, critical swimming speed, U_{crit}, ontogeny.

RESUMEN

La disponibilidad de comida puede afectar la supervivencia larvaria directamente a través del ayuno e indirectamente a través de los efectos sobre la tasa de crecimiento larvario, actividad natatoria y vulnerabilidad a los predadores. En el presente estudio evaluamos los efectos del ayuno sobre el crecimiento, condición nutricional y comportamiento natatorio del lenguado senegalés, *Solea senegalensis,* durante la ontogenia (8 a 14 días post-eclosión). Se llevaron a cabo análisis bioquímicos (RNA/DNA) y experimentos de comportamiento (velocidad crítica de natación, U_{crit}) sobre larvas mantenidas bajo 3 condiciones de alimentación: alimentadas (tratamiento *ad libitum*), ayuno a corto plazo (hasta 48 h sin alimentación) y ayuno a largo plazo (hasta 96 h sin alimentación). El crecimiento varió significativamente según el tratamiento alimentario, mientras que el cociente RNA/DNA y la capacidad natatoria mostraron únicamente una ligera disminución. En los tres tratamientos alimentarios los estadios larvarios más avanzados mostraron velocidades críticas más bajas cuando se compararon con las de los estados de preflexión y flexión, lo que está probablemente relacionado con el estilo de vida bentónica adquirido por la especie al final del periodo larvario. Estos cambios fisiológicos y de comportamiento concuerdan con resultados previos que muestran que las larvas de peces planos son más resistentes al ayuno que de las especies pelágicas y se vuelven menos activas con el desarrollo.

Palabras clave: Lenguado senegalés, *Solea senegalensis,* tratamiento alimentario, ayuno, cociente RNA:DNA, velocidad de natación crítica, U_{crit}, ontogenia

INTRODUCTION

Early life stages of marine fishes experience high rates of mortality, with serious implications on species' future recruitment. The two main mortality agents acting upon marine fish larvae are predation and starvation (Bailey and Houde, 1989). These factors are not independent, as starvation leads to decreased growth rate (Yin and Blaxter, 1986), slower development (Kamler *et al.*, 1990), and changes in behaviour (Sogard and Olla, 1996; Chick and Van den Avyle, 2000). Larvae with low nutritional status will consequently be smaller, weaker and less developed with regard to sensory and locomotory capacities than well-fed larvae of the same age, thus being more susceptible to predation (but see Billerbeck *et al*., 2001). For a variety of fish species (herring, hake, cod, flounder, anchovy, striped bass), it has been shown that starved larvae are more susceptible to predation than fed larvae (Bailey, 1984; Neilson *et al.*, 1986; Yin and Blaxter, 1987; Booman *et al.*, 1991). Several studies indicate that high larval condition can be correlated with increased growth rate and enhanced recruitment or juvenile survival under natural conditions (Searcy and Sponaugle, 2001; Sponaugle *et al.*, 2006), but few attempts have been made to correlate larval condition with any behavioural function critical for larval survival, such as swimming behaviour (e.g. Laurence, 1972; Yin and Blaxter, 1987; Chick and Van den Avyle, 2000). Swimming performance is a central determinant of the fitness of fish, determining to a large extent the success of predator avoidance, prey capture and dispersal potential (Reidy *et al.*, 2000; Armsworth, 2001; Plaut, 2001). Larvae in a better condition may reveal greater swimming abilities and responsiveness to predators than larvae in poor condition (Chick and Van den Avyle, 2000; Grorud-Colvert and Sponaugle, 2006).

Nucleic acid analysis is an acknowledged practical tool to study recent overall nutritional condition and growth of larvae and young fish, as well as their responses to

environmental variability (e.g. Clemmesen *et al.*, 1997; Chícharo, 1997; Buckley *et al.*, 1999; Caldarone *et al.*, 2003). Specifically, RNA/DNA ratio reflects variations in protein synthesis rates: the quantity of DNA in an animal somatic cell is believed to be normally stable, but the quantity of RNA primarily associated with ribosomes, is closely related to the rate of protein synthesis. RNA/DNA ratio has been shown to respond to changes in feeding conditions and growth in periods as short as one to three days in a variety of fish species and is a valid and reliable growth rate estimator (Rooker and Holt, 1996; Buckley *et al.*, 1999; Gwak and Tanaka, 2001).

The present study was undertaken to examine the effect of starvation on growth, nutritional condition and swimming behaviour of laboratory reared Senegalese sole (*Solea senegalensis*) throughout ontogeny, up to the beginning of metamorphosis, when larvae acquire a benthic life mode. We conducted laboratory experiments assessing the critical swimming speed of larvae reared under three feeding treatments (fed *ad libitum*, deprived of food for 2 days and deprived of food for 4 days), and examined growth and nutritional condition, measured as RNA/DNA ratio, of the same tested individuals.

MATERIALS AND METHODS

Larvae

Recently hatched larvae of *Solea senegalensis*, with 4 days after hatching (DAH), were obtained from the Aquaculture Research Station of the National Institute of Biological Resources (INRB-IPIMAR), in Olhão, southern Portugal. Larvae were randomly distributed in five 20 l aquaria, at a density of 100 larvae l^{-1}. Aquaria were filled with filtered seawater, with constant slight aeration and a photoperiod of 12L:12D. Salinity was maintained constant at 37 and temperature average 21ºC. Larvae were assigned to three feeding regimes: fed *ad libitum*, short term starvation (starved for 48 h), and long

term starvation (starved for 96 h). Larvae from the *ad libitum* treatment were stocked in one aquarium (Aquarium 1, Fig. 1) and fed rotifers from 4 to 9 DAH three times per day and *Artemia* nauplii from 6 to 14 DAH three times per day. Food concentration was maintained at a proper density to guarantee an *ad libitum* supply of food. The aeration in the tanks was turned off while feeding, so the larvae didn't lose extra energy on capturing prey. Larvae were fed at least 1.5 h before experiments were conducted. Larvae from the two starving treatments were randomly distributed in four aquaria (see Fig. 1 for experimental design details). Each of the four aquaria provided larvae for both short and long term starvation treatments. Aquaria 2 and 3 supplied larvae twice for the short term starvation treatment. After collecting larvae for the long term starvation experiments (at 11 and 12 DAH, respectively) the remaining larvae in aquaria 2 and 3 were fed once and left starving again for a period of 48 h. Larvae fed *ad libitum* were tested from 8 DAH to 14 DAH; larvae from the short term starvation treatment were tested from 9 DAH to 14 DAH, and larvae from the long term starvation treatment were tested from 11 DAH to 14 DAH.

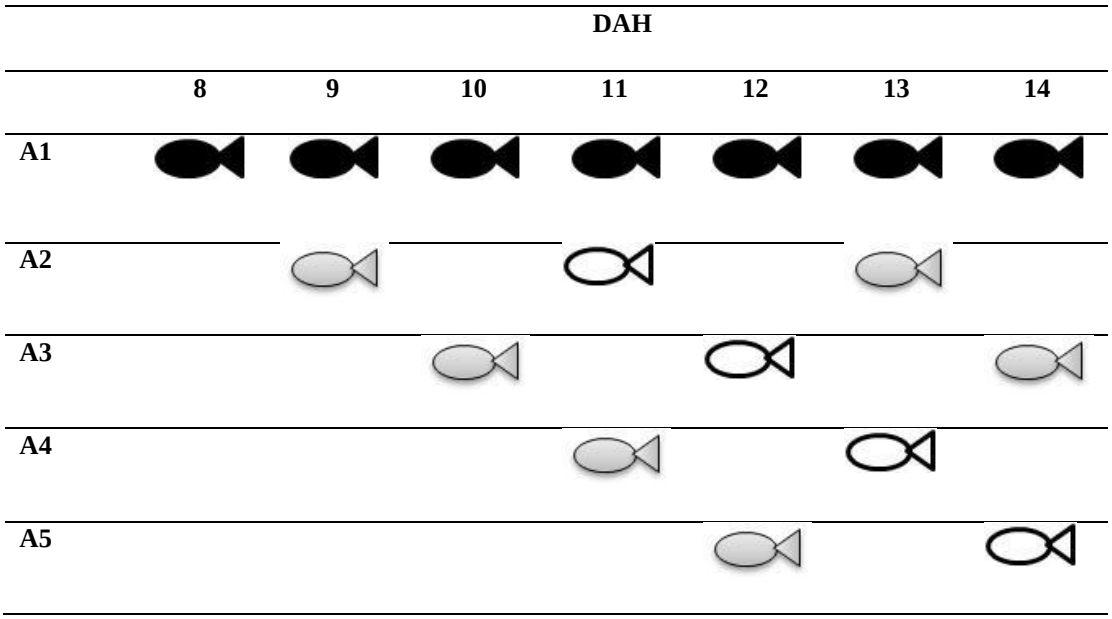

FIG. 1. Experimental design illustrating which aquarium (A1 to A5) supplied larvae to which feeding treatment and ages. Black fish represents larvae from the *ad libitum* treatment, grey fish

represent larvae from the short starvation treatment, and white fish represent larvae from the long starvation treatment.

Experimental design

Larvae were carefully removed from the rearing tank using a small container and placed individually in large petri dishes and left undisturbed to allow recovery from handling (Fuiman and Ottey, 1993). After this period, larvae were transferred to the swimming chamber, one in each lane and allowed to acclimate for 5 min at a minimum flow speed inferior to 1 cm s^{-1}. If any behavioural symptoms of stress, such as lying on the bottom or clinging to the sides, were observed after the acclimation period, the individual was removed and replaced by another fish. Water temperatures in the chamber over the study period varied from 21 to 23ºC.

Critical swimming speed

Critical swimming speed (U_{crit}) was measured using a swimming chamber, following the protocols of Stobutzki and Bellwood (1994, 1997). The chamber was made of clear Perspex with 6 parallel swimming lanes, each 30 mm wide, 50 mm high and 180 mm long. A removable lid allowed introduction and removal of fish from the lanes. A strip of black tape on the top of the lid provided fish with a visual reference to maintain position in the flow, and a mesh screen was placed at the upstream and downstream ends of each lane to retain larvae. A section of flow straighteners, 40 mm long, was placed at the upstream end of each lane to minimize turbulence. Previous work demonstrated that at the typical U_{crit}, water velocity was not significantly different between the centre of the lane and 5 mm from the wall (Stobutzki and Bellwood, 1997; Stobutzki, 1998; Fisher *et al.*, 2000). Experimental observations also confirmed that larvae did not show depth

preference in the chamber. For details on the swimming chamber characteristics see Faria *et al.* (2009). Maximum flow speed achieved reached 22 cm s^{-1}.

To measure U_{crit}, water velocity was increased by approximately 1 cm s^{-1} every 2 min until the larva was unable to swim against the current for 2 min. Calculation of U_{crit} followed Brett (1964):

$$U_{crit} = U + (t/t_i \cdot U_i);$$

where U is the speed of the penultimate increment, U_i is the velocity increment, t is the time swum in the final velocity increment, and t_i is the time interval for each velocity increment (2 min). After the test, fish were immediately put in liquid nitrogen and then stored at -80ºC for subsequent RNA/DNA analysis. The whole larvae were freeze dried and weighed before the biochemical analysis. The whole larvae were freeze dried and weighed before the biochemical analysis. This procedure was selected since, frequently, heads and/or guts are removed for further age and feeding analysis, and these tissues may influence the overall RNA/DNA ratio (Olivar *et al*., 2009).

A total of 215 larvae were tested, however, the number that provided useful data was inferior (n = 161), as each individual did not always provide useful data for all the measured parameters.

Nucleic acid analysis

RNA and DNA were measured with the microplate fluorescent assay (MFA) of Wagner *et al*. (1998). The MFA assay is a modification of the sequential fluorometric method of Bentle *et al.* (1981), in which DNA and RNA in a single sample are determined sequentially by the addition of DNase and RNase, using Ethidium Bromide (EB) as

fluorescent dye (Caldarone *et al.*, 2001). Wagner *et al.* (1998) modified the sequential fluorometric method to the MFA with 96-well microtiter plates by adopting a sarcosyl extraction technique and eliminating the DNase step. The whole larvae were individually homogenized by sonication (3 pulses 50 A during 1 min) with cold sarcosyl extraction buffer. The volume of extraction buffer was 500 µl (0.5%). The samples were then shaken for 1 h at room temperature on a vortex mixer equipped with a multiple-vial head. Next, they were centrifuged (12 000 × g) for 15 min to separate insoluble larvae remains. The samples were diluted 1:10 with Tris buffer to reduce the sarcosyl concentration to 0.05%. In each run, duplicate 50 µl aliquots of supernatants of the samples and duplicates of 0, 0.6, 1.1, 1.7 and 2.3 µg ml^{-1} DNA standard solutions (λ-phagus 0.25 µg $µl^{-1}$ from Roche), 16s–23s *E. coli* RNA (4 µg $µl^{-1}$), 0, 3.6, 7.3, 10.9 and 14.6 µg ml^{-1} RNA standard solutions (16s–23s *E. coli* 4 µg $µl^{-1}$ from Roche) were transferred to 96-well, nuclon, black, round-bottom microplates . The average ratio of DNA and RNA slopes was 5.5 ± 0.8, which can be used to compare RNA/DNA ratio results determined by other protocols (Caldarone *et al.*, 2006). EB solution (30 µl) was added to each well, and the plates were shaken gently at room temperature for 15 min. The EB fluorescence was then scanned on a microplate reader (Biotek synergy HT model SIAFRTD) with 360 nm (excitation) and 590 nm (emission) (first scan- total fluorescence RNA and DNA). Following the first scan, RNase solution (30 µl, 0.12 µg ml^{-1}) was added to each well and the concentration of DNA calculated directly by the standard curve. The concentration of RNA was determinate indirectly by subtraction of DNA fluorescence (second scan) from total fluorescence (first scan).

Statistical analysis

Linear regression analyses were used to determine relationships among size (Standard Length, SL), age (DAH), RNA/DNA ratio and critical swimming speed (U_{crit}) for each feeding treatment (*ad libitum*, short-term starvation and long-term starvation). Normality was tested by using Kolmogorov-Smirnov statistic and plots of residuals and predicted values were examined. The variables SL, U_{crit} and RNA/DNA ratio were $\log_{10}$ transformed to normalize data, but U_{crit} was not normalized by any transformation. Large sample size and the plot of the residuals indicate that analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were robust. To determine whether the slopes of the regressions of size on age differed among the feeding treatments, an ANCOVA was performed with size as the dependent variable, feeding treatment as the fixed factor, and age as the co-variate. Significant effects were further examined by Tukey's HSD multiple comparison tests. To examine the interaction of feeding treatment with ontogeny (pre-flexion, flexion and post-flexion) on mean RNA/DNA ratio and mean U_{crit}, two-way ANOVA were performed after testing for normality and homogeneity of variances. Significant effects were further examined by Tukey's HSD multiple comparison tests. The separate effects of feeding treatment and ontogenetic stage on RNA/DNA ratio and U_{crit} were analyzed by one-way ANOVA. To determine whether the slopes of the regressions of U_{crit} on size differed among the feeding treatments, an ANCOVA was performed with U_{crit} as the dependent variable, feeding treatment as the fixed factor, and size as the co-variate. The relationship between RNA/DNA ratio and U_{crit} was evaluated by regression analysis and an ANCOVA was used to access the effect of feeding treatment on that relationship, with RNA/DNA as the dependent variable, feeding treatment as the fixed factor, and U_{crit} as the co-variate. In all analysis the level of significance was ≤ 0.05. Notes on ontogenetic progress were made and SL was measured

to the nearest 0.01 mm, using the Image J software (version 1.38). Before each photograph was taken, a transparent acetate sheet marked with a millimetre grid was photographed and used as a reference before each measurement in the image analysis software. All statistical tests were conducted using STATISTICA software (Version 6.0).

RESULTS

Effect of starvation on growth and RNA/DNA ratio

Growth in SL of *Solea senegalensis* was significantly influenced by feeding treatment ($F_{(2,157)} = 29.61$, $p<0.0001$). Larvae reared under the long-term starvation treatment were significantly smaller than larvae reared under the other two treatments (Fig. 2).

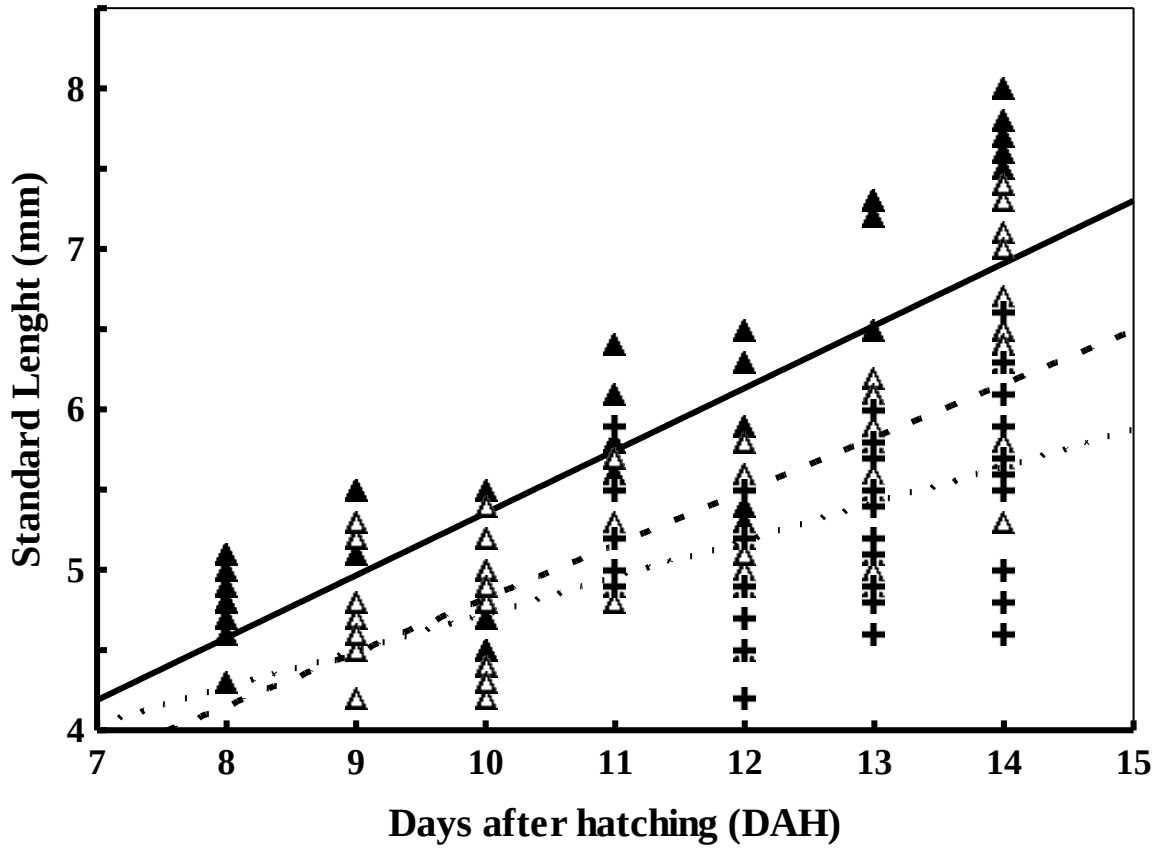

FIG. 2. Changes in standard length (SL) of fed and starved larvae of *Solea senegalensis*. Each symbol represents results for an individual larva. Closed triangles represent fed fish ($y = 0.39x + 0.94$, $r^2 = 0.64$); opened triangles represent short term starvation fish ($y = 0.33x + 0.98$, $r^2 = 0.56$), and cross symbols represent long term starvation fish ($y = 0.23x + 1.94$, $r^2 = 0.17$). The thick line

is the regression line for fed fish; the broken line is the regression line for short term starvation fish and the dotted line is the regression line for long term starvation fish.

RNA and DNA contents of 56 fish of the fed group, 66 fish of the short-term starvation group and 39 fish of the long-term starvation group were analyzed (Table 1). The interaction between feeding treatment and ontogeny returned no significant effects ($F_{(4,152)}$ = 0.33, p = 0.86). RNA/DNA ratio did not change with ontogeny ($F_{(2,158)}$ = 1.10, p = 0.33), and a long-term starvation period (96 h) was not enough to significantly decrease nutritional condition ($F_{(2,158)}$ = 2.02, p = 0.13).

TABLE 1. Number of larvae, age range, mean size (±SE) and mean nucleic acid content of analyzed larvae (±SE) in the three feedingtreatments. N = number of larvae, DAH = days after hatching, SL = standard length, W = weight, DW = dry weight, SE = standard error.

	N	DAH	SL (mm)	W (mg)	µg RNA/larva	µg RNA/mg DW	µg DNA/larva	µg DNA/mg DW	RNA/DNA
Ad libitum	56	8-14	5.18±0.13	0.59±0.05	15.54±2.83	36.30 ±6.08	12.58±1.47	27.69±3.23	1.32±0.14
Short term	66	9-14	4.86±0.09	0.40±0.03	13.44±2.40	39.45±5.88	11.91±1.15	40.91±4.38	1.56±0.15
Long term	39	11-14	4.89±0.10	0.47±0.05	12.43±3.51	39.25±10.54	12.43±0.80	42.53±6.70	0.91±0.19

Effect of starvation on Critical speed

A total of 161 larvae were tested in critical swimming experiments, of which 56 belonged to the fed group, 66 to the short-term starvation group and 39 to the long-term starvation group. Of these, 22 larvae of the fed treatment, 17 larvae of the short-term starvation treatment and 13 larvae of the long term-starvation treatment did not swim either because they were pre-flexion stages, incapable of swimming against the slowest tested current speed, or post-flexion stages, metamorphosing and settled to the bottom. These larvae were assigned a critical speed of 0 cm s^{-1}.

Critical speed of the fed and short-term starvation treatments revealed a significant relationship with size (fed: $F_{(1,54)} = 7.12$, $p = 0.01$; short-term starvation: $F_{(1,64)} = 10.96$, $p = 0.001$), but no significant relationship in larvae of the long-term starvation treatment was registered ($F_{(1,37)} = 0.16$, $p = 0.69$) (Table 2).

TABLE 2. Summary of measurements of critical swimming speed (cm s^{-1}) for *Solea senegalensis* in the different feeding treatments: Number (N), size (SL: standard length), age (DAH: days after hatching), relationships between critical speed (U_{crit}: log_{10} transformed) and size (SL: log_{10} transformed SL). Not significant $p>0.05$.

	N	SL (mm)	Age (DAH)	Relationship	R^2	p	Range
Ad libitum	56	3.5-7.5	8-14	y = -0.80x + 0.74	0.12	0.01	0.0-5.0
Short term	66	3.7-6.9	9-14	y = -0.91x + 0.81	0.15	0.001	0.0-5.0
Long term	39	3.7-6.1	11-14	y = -0.19x + 0.32	0.004	0.69	0.0-3.2

The interaction of feeding treatment with ontogenetic stage returned no significant effect on U_{crit} ($F_{(4,152)} = 0.59$, $p = 0.67$). Feeding treatment had no influence on U_{crit} ($F_{(2,158)} = 0.13$, $p = 0.88$), with performance ranging from 0 to 5 cm s^{-1} in fed and short-term starvation larvae, and 0 to 3.2 cm s^{-1} in long-term starvation larvae. Ontogenetic stage had a significant effect on mean U_{crit} values ($F_{(2,158)} = 14.70$, $p<0.0001$). Tuckey-test revealed

that post-flexion larvae had a significantly slower swimming performance when compared to earlier developmental stages (Fig. 3).

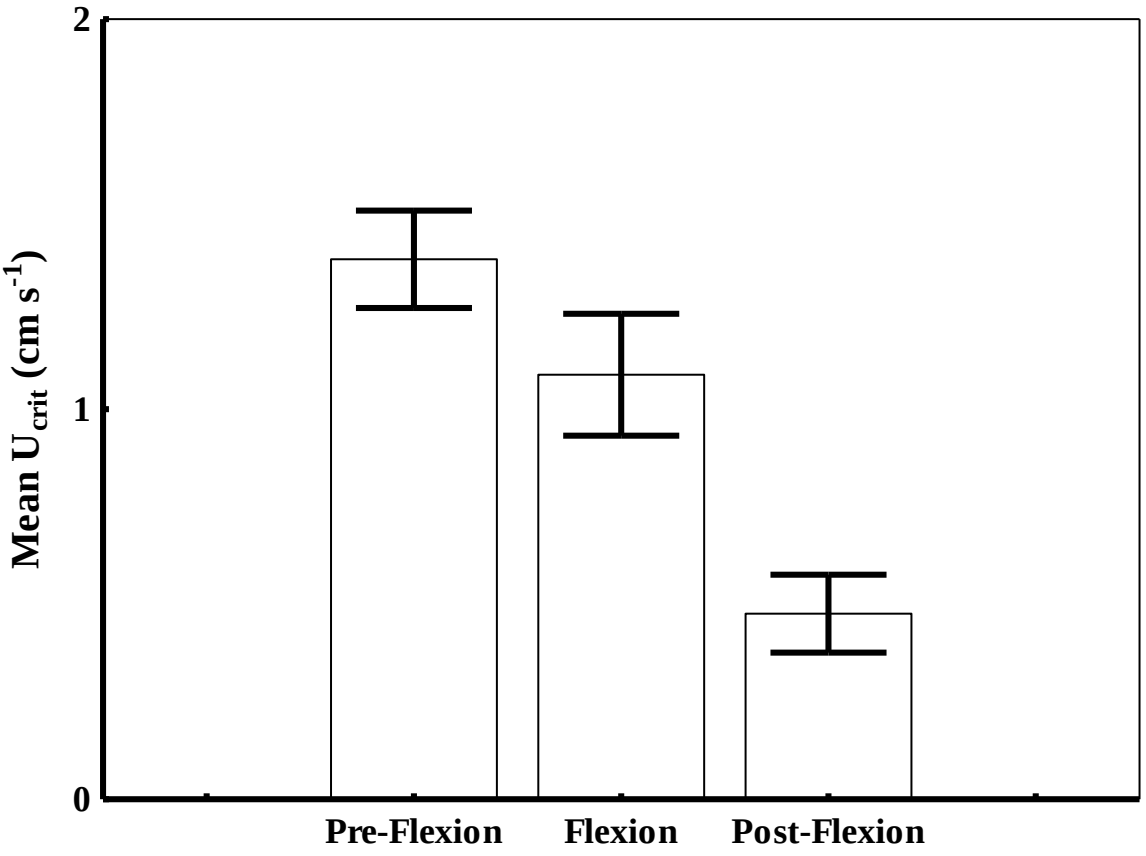

FIG. 3. Mean U_{crit} (± SE) of *Solea senegalensis* larvae in pre flexion, flexion and post flexion stages.

The relationship between RNA/DNA and U_{crit} did not change with ontogenetic stage ($F_{(2,157)} = 1.90$, $p = 0.15$), and showed no differences between the feeding groups ($F_{(2,157)} = 2.09$, $p = 0.13$) (Fig. 4). Also, the regressions of RNA/DNA on U_{crit} were not significant in any feeding treatment (fed: $F_{(1,54)} = 1.12$, $p = 0.29$; short-term: $F_{(1,64)} = 3.73$, $p = 0.06$; long-term: $F_{(1,37)} = 2.06$, $p = 0.16$).

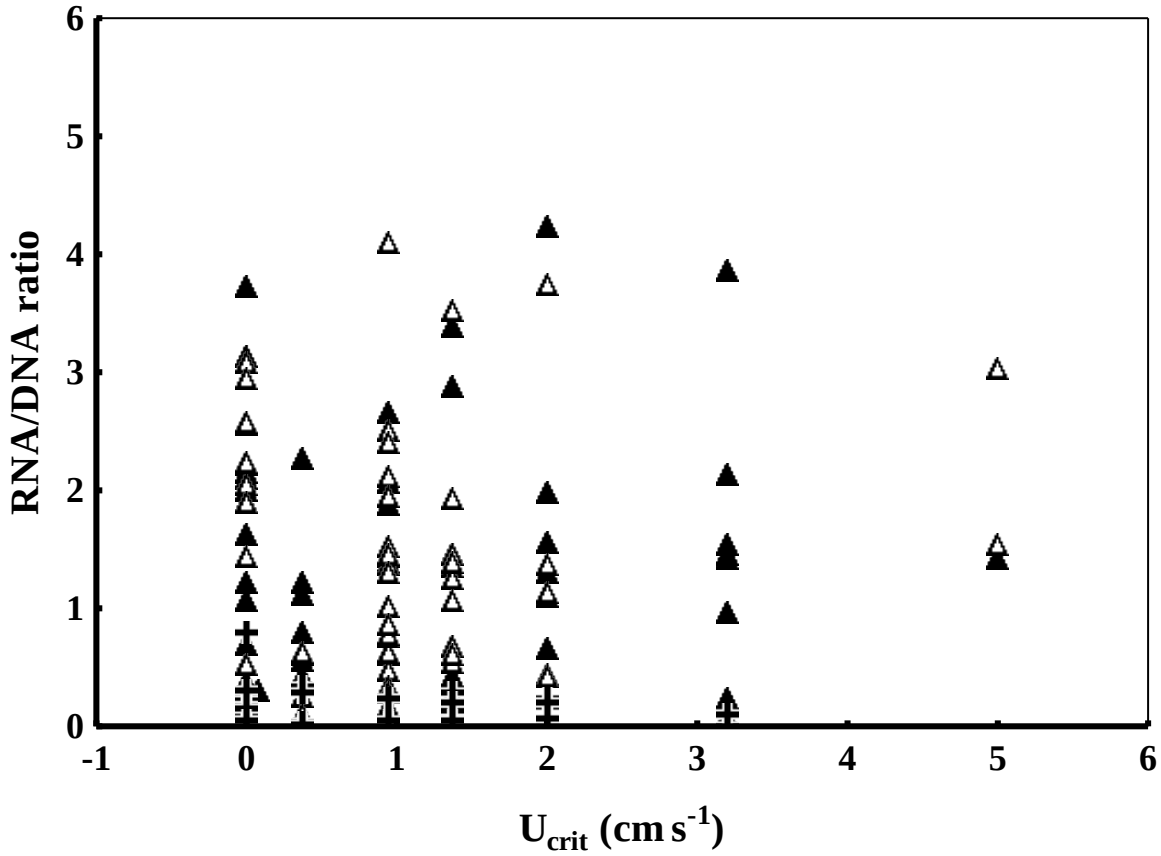

FIG. 4. Relationship between RNA/DNA ratio and U_{crit} of fed and starved larvae of *Solea senegalensis*. Closed triangles represent fed fish, opened triangles represent short term starvation fish, and cross symbols represent long term starvation fish. The x-axis is scaled to start at -1 instead of 0, for a better visualization of the graph.

DISCUSSION

Effect of starvation on growth and RNA/DNA ratio

Starvation had a significant influence on growth of *Solea senegalensis* larvae, with 4 d starvation larvae showing considerable slower growth when compared to fed and 2 d starved larvae. Reduced growth rate caused by starvation will extend the duration of vulnerable ontogenetic stages, and as there are more potential predators for small larvae, starved larvae might suffer a higher cumulative rate of mortality (Cushing, 1975; Shepherd and Cushing, 1980). Survival of starved larvae is then compromised. The results of nutritional condition, measured as RNA/DNA ratio, showed a slight decrease

after 4 d of starvation. Latency has been demonstrated to be as short as 1-3 days in some fish species. Clemmesen (1994) reported that fed and starved herring (*Clupea harengus*) larvae (>10 d) could be distinguished only after 3 to 4 days. Other than that, it has been shown that flatfish larvae are more resistant to starvation (Clemmesen, 1987; Yin and Blaxter, 1987) when compared to species with small eggs and yolk-sac larvae, such as *Engraulis mordax* (Lasker *et al.*, 1970), *Paralichtys californicus* or *Hypsopsetta guttulata* (Gadomski and Petersen, 1988), and to species carrying larger yolk reserves, such as *Clupea harengus* (Blaxter and Hempel, 1963) or *Ammodytes americanus* (Buckley *et al.*, 1984).

Richard *et al.* (1991) studied the effect of starvation on RNA/DNA ratio in *Solea solea* larvae and concluded that the time of beginning of starvation was an important factor. Larvae starved from the beginning of exogenous feeding showed a sharp decrease in the RNA/DNA ratio, and could be distinguished from fed larvae after 2 or 3 d of starvation. But for larvae starved after days 5 and 10, the food deprivation interval must be longer to show a clear effect, and in juveniles starved Day 14 after metamorphosis, the RNA/DNA ratio was not significantly different from that for fed juveniles throughout the experiment (Richard *et al.*, 1991).

Despite studies on how larval condition affects behaviour, in particular swimming performance and escape response (Laurence, 1972; Yin and Blaxter, 1987; Chick and Van den Avyle, 2000; Grorud-Colvert and Sponaugle, 2006), there is no study relating swimming behaviour and nutritional condition measured as RNA/DNA ratio. RNA/DNA ratio has been shown to respond to changes in feeding conditions and growth in periods as short as one to three days in a variety of fish species and is a valid and reliable growth rate estimator, which has been applied in numerous field assessments (e.g. Rooker and Holt, 1996; Buckley *et al.*, 1999; Gwak and Tanaka, 2001; Chícharo *et al.*, 2003), but has

never been correlated with any behavioural function. In this study, RNA/DNA ratio did not correlate significantly with critical speed, which may be related to the high resistance to starvation and low swimming performance of *Solea senegalensis* larvae.

Effect of starvation on Critical speed

Swimming abilities of larval fish are critical behavioural traits since swimming influences the capacity of larvae to find food, escape from predators and control dispersal (Stobutzki and Bellwood, 1994, 1997). In order to determine the potential importance of swimming behaviour, it is fundamental to know how these abilities change during ontogeny (e.g. Leis *et al.*, 2009a,b; Faria *et al.*, 2009). In the present study, a significant decrease on swimming abilities of post-flexion larvae was observed in the three feeding treatments, which implies an ontogeny-related effect. The lack of swimming or reduced swimming of larvae close to settlement is probably related to behavioural changes associated with a benthic lifestyle, and not a decrease in the larval abilities to swim. These larvae were completing metamorphosis and resembled newly settled fish. Behavioural changes coupled with morphological changes are known to lead to variation in the general metabolism (Bergeron, 1982) and energy expenditure during flatfish later development, when flatfish larvae are less active (Blaxter and Staines, 1971). Other studies have also related changes in swimming performance to developmental or ecological transitions (e.g. Dudley *et al.*, 2000; Guan *et al.*, 2008), reporting an improvement in swimming performance until metamorphosis, after which improvements became slower. Opposite of what was found in other studies (Clark *et al.*, 2005; Leis *et al.*, 2006a,b, 2007, 2009a,b; Faria *et al.*, 2009), there was no significant relationship between critical speed and size, in either feeding treatments. This can be attributed to the small size range of tested larvae, or merely indicative of the poor swimming capacities of *Solea senegalensis* larvae. Critical

swimming speed recorded for Senegalese sole larvae in this study ranged from 0.4 to 5.0 cm s^{-1} (0.2 to 11.9 bl s^{-1}) for fed and short-term starvation larvae, and 0.4 to 3.2 cm s^{-1} (0.6 to 7.3 bl s^{-1}) for larvae starved for 4 d. There are no data on critical speed of other Pleuronectiformes, but data available for routine speeds of Soleidae, Pleuronectidae and Paralichthyidae report values of 0.5 to 3 cm s^{-1} (Blaxter, 1986; Miller *et al.*, 1988), which are close to what we found in this study. These values are in agreement with the typical assumption that temperate fish larvae are poor swimmers. This assumption can owe, in part, to differences in water temperature. It is well known that temperature influences both the physiology of fish larvae and the physics of the hydrodynamic environment in which larvae are swimming (Fuiman and Batty, 1997). Other than these, variation can result from morphological differences (Stobutzki, 1998; Dudley *et al.*, 2000). Most studies compare temperate clupeiform, gadiform, or pleuronectiform larvae (Blaxter, 1986; Miller *et al.*, 1988) with tropical perciform larvae (Fisher *et al.*, 2000, 2005; Leis and Fisher, 2006). For this reason, comparisons of swimming speed among taxa should take into account phylogeny, methodology, and developmental state.

No food deprivation-induced effects in swimming behaviour were found, after a 4 d period of food deprivation, suggesting that there are no condition-related behavioural effects in critical swimming performance after 4 d of starvation. Regarding escape response behaviour, Skajaa and Browman (2007) also concluded that escape response rate of cod larvae deprived of food for 3 d was not affected. In contrast, Yin and Blaxter (1987) found condition-related behavioural changes, with the escape response rate presenting a dome-shaped relationship with increasing duration of food deprivation: escape speed in herring (*Clupea harengus*) of different ages, and newly hatched cod and flounder (*Platichthys flesus*), initially increased with increasing time of food deprivation, peaked when the larvae were close to the point of no return (PNR, when 50% of the

larvae will die even when offered food), and subsequently decreased. The escape response rate has also been shown to have a negative relationship with increasing duration of food deprivation. Booman *et al.* (1991) reported a decrease in responsiveness of starved northern anchovy (*Engraulis mordax*) larvae to predatory attacks by adult anchovy. Chick and Van den Avyle (2000) examined the effects of feeding on routine swimming speed and responsiveness to simulated predator attacks of larval striped bass (*Morone saxatilis*) reared under high, medium and low prey treatments and found that larvae reared in the low-prey treatment had slower routine swimming speeds and shorter reactive distances and were less responsive to simulated-predator attacks. The effects of starvation were not evident until larvae had starved for several days. Results then suggest that susceptibility to starvation of fish larvae appears to be species-specific.

Overall, our results indicate that growth was significantly affected by feeding treatment, but it registered only slight decreases on RNA/DNA ratio and swimming performance after 4 days of starvation. These behavioural and physiological changes are in accordance with previous results, which show that flatfish larvae become less active later in development and are more resistant to starvation compared to pelagic species. In the future it will be imperative to have information on wild larvae, to allow inferences of the observed behaviours to their ecology and life history patterns (Leis, 2006).

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CHAPTER V. GENERAL DISCUSSION

1. Larval Descriptions

Most ecological studies involving early life-history stages of fish require accurate identifications of eggs and larvae. However, the identification of fish eggs and larvae collected in plankton samples is no easy task and, for this reason, many studies on larval fish assemblages provide data mostly to the family level (e.g. Kingsford and Choat 1989, Brogan 1994, Palomera and Olivar 1996, Sabatés et al. 2003). In many instances, this prevents the investigation of species-specific ecological patterns. The lack of complete descriptions of larval stages and their ontogenetic development is particularly notorious as far as reef species are concerned (e.g. Gobiidae, Blenniidae, Labridae, Gobiesocidae). Few reef species are of major commercial importance, so they have largely escaped the attention of fishery biologists.

For Gobiesocidae larvae, for example, the descriptions available are incomplete (see Guitel 1888, Padoa 1956, Russel 1976). In particular, the two species for which the developmental stages are described in **chapter II** (*Lepadogaster lepadogaster* and *L. purpurea*) were misidentified until very recently (Henriques et al. 2002). Consequently, the larval developmental studies of *Lepadogaster* genus made until then (Guitel 1888, Russell 1976, Padoa 1956) were confused and useless in identifying both species correctly, since the developmental stages were all mixed into a single species.

The direct approach method (Miller and Kendall 2009) was used to identify and describe larval development of the two above mentioned gobiesocids (**chapter II**). This method involves rearing larvae from parents of known identity. Larvae were described at various stages of development, which will allow identification of wild-caught larvae by comparing them with the descriptions of the reared larvae (Miller and Kendall 2009). Larvae of both species hatched with 5.2 mm in length, with the mouth and anus opened, eyes pigmented, opercula opened and very little yolk. This advanced developmental

stage at hatching is typical of marine fishes with male parental care (Thresher 1984, Sponaugle et al. 2002, Hickford and Schiel 2003) that spawn demersal eggs. Larval development lasted 33 days in *L. purpurea*, at a mean temperature of 14.5 ºC, while *L. lepadogaster* larval development was faster, lasting 18 days, at a mean temperature of 16.5 ºC. The pelagic larval duration (PLD) found in this study for *L. lepadogaster* (18 days) is in accordance with the estimated period of larval duration described by Beldade et al. (2007), based on otoliths readings of new settlers collected in the field. Although there are no studies on PLD of *L. purpurea*, laboratory rearing suggests that PLD is approximately 30 days at a temperature of 14.5 ºC.

Fish larvae possess a large suite of characters used for taxonomic purposes, such as body morphology, pigmentation, meristics and specialized larval characters (Miller and Kendall 2009). The larval pigmentation usable as a taxonomic character is limited to melanophores, since the other pigmented cells (e.g. xanthophores) do not retain their color after preservation (Miller and Kendall 2009). The relative size, position, and sometimes the number of melanophores in series should be noted. In the case of the studied gobiesocids (**chapter II**), pigmentation was the only trait that could be used to distinguish between the larvae of the two species. The absence or residual pigmentation on the ventral region of *L. purpurea* as opposite to the presence of several pigments on the ventral region of *L. lepadogaster*, from the lower jaw till the anus, allowed the correct identification of larvae of these species throughout the larval period.

The basic sequence of larval developmental events agreed largely between the two species. However, the timing of events was different due to the larger pelagic duration of *L. purpurea*. The change to a benthic mode of life was gradual in both species, with larvae increasingly spending more time close to the bottom until definitely settling.

Despite being time consuming, labor intensive and not always successful, rearing larvae from eggs of captive broods is a good way to allow valid descriptions of larval stages. However, data from reared larvae should be used with caution since laboratory and field conditions may vary extensively. Reared larvae can often differ from field-caught larvae concerning pigmentation, morphological and meristic characteristics (Leis 2000). The pelagic larval duration (PLD) and growth rates may also differ between reared and wild larvae. Temperature is known to greatly influence larval duration and growth rates. In temperate waters, the increase in growth rate of fish larvae observed in the spring and the decrease observed in the fall has most often been attributed to or correlated with water temperature (Campana and Hurley 1989, Munk et al. 1991). Attribution of increased growth to water temperature in the field is also supported by numerous laboratory experiments that demonstrate faster larval growth rates at higher temperatures both within and among species (Pepin 1991, Houde and Zastrow 1993). However, considering that the rearing temperature used in larval developmental studies described in **chapter II** followed the temperature range observed in the field, it is unlikely that this factor would have been responsible for major differences in PLD observed in the present work.

Photoperiod is also known to influence the length of the larval period and growth rates of reared reef fish larvae. Arvedlund et al. (2000) studied the effect of photoperiod on growth of larvae and juveniles of the anemonefish, *Amphiprion melanopus*, and found that larvae reared under 24L:0D (Light:Darkeness) had faster growth rates than larvae reared under 12L:12D photoperiod regime, but lower growth rates than larvae reared under a 16L:8D regime (Arvedlund et al. 2000). The authors suggest that fish in the extended light regimes feed for longer periods of time than those reared under 12L:12D photoperiod, thereby yielding higher rates of growth and development. The fact that

growth under 24L:0D was slower than under 16L:8D suggests that unless the developing larvae have a period of inactivity during darkness, their growth is compromised. In contrast, Kiyonon and Hirano (1981) reported an optimum growth rate of black porgy (*Mylio macrocephalus*) with continuous light, and Tandler and Helps (1985) also reported this for gilthead sea bream (*Sparus aurata*). The studies of larval development described in **chapter II** were conducted under a 24 hour light regime, which is a common procedure to maximize larval growth and survival in rearing conditions. Since most marine fish larvae are visual feeders (Blaxter 1986), photoperiod determines the time available for feeding and consequently has a considerable impact on daily ingestion rates in marine fish larvae (Suthers and Sundby 1996). Different results could be expected if larvae were reared under alternate light and darkness regimes. Despite this cautious, it appears that this photoperiod did not induce changes in the duration of the larval period of *L. lepadogaster*, since the PLD of reared larvae was well within the values reported for wild larvae (Beldade et al. 2007) and other spring-spawners, temperate gobiesocids (e.g. *Apletodon dentatus* = 15 days, *Gouania wildenowi* = 17 days, *Lepadogaster candollei* = 13 days; Raventós and Macpherson 2001). There are no data available regarding pelagic duration of wild *L. purpurea* or other winter-spawner gobiesocids, and therefore it is not prudent to state that the photoperiod used for rearing larvae did not affect growth rates and pelagic duration. This issue deserves further studies.

In the future, otolith microstructure analysis could be used to clarify differences between laboratory-reared larvae and wild-caught gobiesocid larvae for validation of data (e.g. Jones and Brothers 1987, Vigliola 1997, Aldanondo et al. 2008). Since the discovery of daily ring deposition in otoliths, daily increments have been extensively used to determine age (e.g. Campana and Neilson 1985, Jenkins 1987), size-at-

settlement (e.g. Victor 1991, Raventós and Macpherson 2001, Thorrold and Hare 2002, Hamilton and Warner 2009) and growth rates, providing relevant results to the understanding of recruitment patterns (e.g. Searcy and Sponaugle 2000, Meekan et al. 2003, Sponaugle and Pinkard 2004, Raventós and Macpherson 2005, Sponaugle et al. 2006).

Larval rearing also helps elucidating important processes throughout larval development. The detailed descriptions of larval development facilitate correlations of morphological development to behavioural development (Murphy et al. 2007). For example, Clark et al. (2005) studied the swimming ontogeny of four temperate marine reef fishes and correlated the significant improvement in swimming behaviour to the important morphological events occurring at that time, such as the completion of notochord flexion and caudal fin formation. Fisher et al. (2000) studied the development of swimming ability in larvae of *P. amboinensis* in the laboratory, and portrayed a marked increase in swimming speed at 7–9 mm standard length (SL). Morphologically, there was no major development event; however, between 7 and 9 mm, relative body depth increased by one third, from c. 30 to 40% SL, resulting in a large increase in muscle mass, justifying the marked increase in swimming performance in this species. **Chapters III and IV** describe the development of swimming abilities of reef species whose larval development is already known and, therefore, improvement of swimming performance could be correlated with morphological events occurring during ontogeny. For most studied species, the improvement of swimming performance was coincident with morphological changes, such as completion of notochord flexion (**III-A, IV-A**) and radical changes in body shape and structure caused by a significant allometric growth (**III-B**).

2. Ontogeny of Swimming Behaviour of Temperate Reef Fishes

The survival of individuals at any stage of life requires adequate levels of performance against a variety of ecological challenges, such as obtaining food, evading predators, and locating and remaining in a suitable habitat (Fuiman 2002). Meeting these challenges is especially difficult during early life because larvae are so small and incompletely developed. Ontogeny and growth during early life make this an especially dynamic period during which new capabilities arise and performance levels improve rapidly (Fuiman 2002). In this context, the onset of development of swimming capabilities assumes major importance to larval survival and transport.

Most swimming behavioural studies have focused on larvae nearing the end of their pelagic period when they are already competent to settle, and only limited information on the ontogeny of these behaviours is available (but see Fisher et al. 2000, Clark et al. 2005, Leis et al. 2006a b, 2007, 2009a, b, **chapters III-A, B**). However, in order to understand the potential importance of active swimming of fish larvae, information on swimming ability is needed for the entire larval period, as it is unrealistic to assume that behavioural abilities midway through development would be similar to those at the end of the pelagic development (Leis et al. 2009b).

Ontogeny of swimming speed was examined herein for four families of fishes, belonging to three different orders: Perciformes (**III-B** and **IV-A**), Gobiesociformes (**III-A**) and Pleuronectiformes (**IV-B**). For all these species, critical swimming speed (U_{crit}) was the common measure of swimming performance. Perciforms (gilthead seabream and red drum) were better swimmers, with similar performances and maximum critical speeds up to 20 cm s^{-1} close to settlement size. The pleuronectiform (senegalese sole) was the poorest swimmer, with speeds not exceeding 5 cm s^{-1} right before metamorphosis. Gobiesociforms had an intermediate performance, with critical

speeds up to 6 and 9 cm s^{-1} (depending on the species) when approaching settlement size. For all the species, except senegalese sole, critical speed increased with size, although at a species-specific rate. All studies found a large variation in swimming performance at any size. We chose to use critical swimming speed to assess swimming ability mainly because U_{crit} has proven useful in understanding dispersal, recruitment and connectivity of reef fishes (Stobutzki 1997, Stobutzki and Bellwood 1997, Bellwood and Fisher 2001, Green and Fisher 2004, Leis and Fisher 2006, Grorud-Colvert and Sponaugle 2006). U_{crit} has also been shown to be positively correlated with other ecologically relevant measures of swimming ability, such as routine swimming speeds (Fisher and Bellwood 2003), sustainable swimming speeds (maintainable for 24 hours) (Fisher and Wilson 2004), and *in-situ* speed (Leis and Fisher 2006, Leis et al. 2006a); and a recent review (Plaut 2001) concluded that U_{crit} is the best ecophysiological measure of swimming performance in fishes because it can be correlated to other more ecologically relevant traits.

Swimming performance affects every aspect of a fish's ecology, from the acquisition of food and avoidance of predators (Videler 1993), to successful migration (Fisher and Bellwood 2003), ultimately influencing Darwinian fitness (Plaut 2001, Reidy et al. 2000). Although not necessarily the only factor of importance in fish swimming (physiological factors such as muscle type must also be considered), body morphology is clearly highly correlated with swimming performance and therefore changing size and structure throughout development strongly influences swimming performance (Webb 1984). The gradual appearance of fin rays and reduction of the median fin fold are obvious changes to the development of a larva's swimming apparatus. Gobiesocids (**III-A**) showed a greater swimming performance after completion of flexion, which is coincident with the formation of the caudal structures. In many species, the caudal fin

displays an exceptionally high degree of positive allometry during the larval period (Fuiman 1983, Peck et al. 2005, Peña and Dumas 2009), which results in a rapidly increasing propulsive area (Fisher et al. 2000). High allometric growth also occurs in the region of the caudal peduncle, signaling disproportionate growth of locomotor muscle mass (Fuiman 1983). In red drum (**III-B**), the improvement of swimming performance was coincident with the radical changes in body shape and structure caused by a significant allometric growth. The muscles themselves undergo important changes, starting with a single dominant fiber type and only later developing the two-gear system of red and white muscle (Batty 1984, Patruno et al. 1998). These ontogenetic changes, together with actual scaling effects, result in the gradual improvement of swimming performance as larvae develops (Fuiman 2002), which has relevant ecological impacts.

2.1 Ontogeny of Swimming Speed – Temperate Perciformes

Not surprisingly, the studied temperate perciform species (*Sciaenops ocellatus* - **III-B**, and *Sparus aurata* - **IV-A**) became more competent swimmers with growth, although variation at any size was large. Rates of speed increment with growth were quite similar between species (0.4 to 4.2 cm s^{-1} per mm growth for gilthead seabream, *Sparus aurata*, and 0.5 to 3.7 cm s^{-1} per mm growth for wild red drum, *Sciaenops ocellatus*), and are in agreement with the results reported for other pelagic warm-temperate sciaenid and sparids (1.2 to 2.6 cm s^{-1} per mm growth, Clark et al. 2005) and temperate sparids (0.1 to 3.7 cm s^{-1} per mm growth, Pattrick and Strydom 2009).

Nevertheless, the ontogenetic increase in critical swimming speed differed between species. In gilthead seabream, critical speed increased throughout development, and no changes in performance were observed, probably due to the small size range used (6-14 mm SL) and also due to the fact that larvae could not be tested until the settlement

phase (< 20 mm TL; Koumoundouros et al. 2009). In contrast, in red drum, swimming ability advanced in two phases, with a sharp change in the trajectory of swimming performance coincident with important changes in ecology, morphology, and hydrodynamics (**III-B**). There was an early phase of more rapid improvement which changed to a later phase of slower improvement when the larvae attained 6.9 mm TL, which is approximately the size at which red drum leave the water column and settle into seagrass beds (Rooker et al. 1998). Thus, the swimming ability of red drum larvae increases more rapidly during the pelagic (pre-settlement) stage than the post-settlement stage. In terms of morphology, larvae smaller than 6.9 mm are also subject to radical changes in body shape and structure. Specifically, growth profiles showed that the head and caudal regions grow faster than the other parts of the body at these smaller sizes. At the breakpoint between the two phases of ontogenetic increase in critical swimming speed, red drum larvae attained a Reynolds number of 640. Under these conditions, they enter the intermediate hydrodynamic regime (Sarkisian 2005) and begin to escape the energetic burden of the viscous hydrodynamic regime at the same time that highly allometric growth produces a body shape that is suited to efficient locomotion in an inertial hydrodynamic environment.

Previous studies have correlated changes in swimming performance to developmental or ecological transitions, but pattern of changes are not consistent across species. When studying sustained swimming abilities of reef fish larvae from New Zealand, Dudley et al. (2000) reported specimens of *Upeneichthys lineatus* (Mullidae) that did not swim or decreased swimming abilities after settlement due to changes associated with a benthic life style. The authors suggested that this decrease was due to a habitat shift associated with settlement. These results agree with the ontogenetic trend found for red drum larvae (**III-B**). On the contrary, Fisher et al. (2000) found that the swimming ability of

coral reef fish larvae increased more slowly in the early life stages and faster in later stages, and Clark et al. (2005) found the same trend when studying critical and endurance swimming performance of four species of temperate marine fishes (Sciaenidae, Sparidae, Percichthyidae). These authors suggest that improvements in swimming performance occur markedly after notochord flexion was complete. After notochord flexion, the larvae were clearly capable of swimming in an inertial environment and it was at this point that endurance and critical speed abilities started to become substantial.

Despite the already discussed differences in the ontogeny of critical swimming speed, red drum and gilthead seabream had similar swimming abilities. U_{crit} in reared red drum larvae ranged from 1.1 to 20.5 cm s^{-1}, over the size range of 3 to 19.1 mm TL, while U_{crit} of reared gilthead seabream ranged from 3 to 19.3 cm s^{-1}, over the size range of 6.2 to 14.1 mm SL. These values are well within the speeds reported for other temperate and warm temperate perciform species. Koumoundouros et al. (2009) have, as well, examined the ontogeny of critical swimming speed of premetamorphic (13.7-18.7 mm total length, TL) and postmetamorphic (20.4-34.3 mm TL) *S. aurata* larvae at different temperatures (15, 20, 25 and 28 °C). At 20 ºC, and at early metamorphosis, average relative U_{crit} of larvae was 6.2 bl s^{-1} (body length, Koumoundouros et al. 2009), which is notably lower than average relative speeds (10.5 bl s^{-1}) reported in our study. Additionally, our larvae were, on average, much smaller (4.8-15.7 mm SL) than the ones used in the Koumoundouros et al. (2009) study. The differences in observed swimming speed may also be attributed to the experimental protocols themselves, as the swimming chambers differed between studies, as did the speed increments and time intervals used between each increment. Although Hogan et al. (2007) concluded that U_{crit} is relatively robust to variations in methodologies (with respect to length of the

time interval employed), it is fundamental to standardize procedures for such experiments, in order to allow meaningful comparisons among studies, regardless of methodologies. The experimental set-up used in our studies followed the set-up first proposed by Stobutzki and Bellwood (1994, 1997), which has been adopted ever since for these type of protocols.

Recently, Pattrick and Strydom (2009) examined U_{crit} and endurance abilities of late-stage wild larvae of two other temperate Sparidae, *Diplodus capensis* and *Sarpa salpa*, using an experimental set-up similar to ours, and reported maximum U_{crit} values of 35 and 33 cm s^{-1}, respectively. These speeds are greater than the ones recorded in the present study for *Sparus aurata*. However, the larvae in Pattrick and Strydom (2009) study were larger (8.9-21.3 mm) than larvae in our study (4.8-15.7 mm SL), which could account for these differences in maximum U_{crit} speed. Clark et al. (2005) studied the ontogeny of U_{crit} of two other warm-temperate Sparidae, *Acanthopagrus australis* and *Pagrus auratus*, over a size range very close to the size range used in the present work for *Sparus aurata*, and U_{crit} speeds varied between 2 and 27 cm s^{-1}, which falls within the U_{crit} range here described. Clark et al. (2005) have also examined the ontogeny of swimming of a sciaenid, the mulloway (*Argyrosomus japonicus*) (Clark et al. 2005). Maximum U_{crit} for this species was 16.6 cm s^{-1}, at 10 mm SL (Clark et al. 2005), which is lower than the maximum U_{crit} reported for red drum (22.2 cm s^{-1}).

The ontogeny of routine speed – the mean rate of travel during undisturbed activity (Fuiman et al. 1999) – has also been examined for gilthead seabream (**IV-A**). Average routine speeds of larvae in the experiments were low, ranging from 0.9 to 17.2 mm s^{-1}, which represents *ca*. 4 to 8 % of their U_{crit}, respectively. As opposite to other studies on routine speed (e.g. Fuiman and Webb 1988, Fuiman et al. 1999, Smith et al. 2004), in

our study no significant relationship between routine swimming speeds and length or age was observed. Chick and Van Den Avyle (2000) have also found a poor relationship between routine speed and length for striped bass (*Morone saxatilis*) larvae, and attributed the poor relationship to the relatively narrow range of age and size tested. This could also explain the results obtained in chapter **IV-A** for gilthead seabream larvae, which were tested between 6.4 and 15.7 mm (SL). Despite the absence of a linear relationship between routine speed and length, the general trend showed a bell-shaped curve, with speed increasing to a certain size, after which it started to decrease. This reduced swimming activity after a certain size (10 mm SL, in the present study) might be related to behavioural changes associated with a benthic lifestyle, since these were pre-metamorphosing larvae (settlement size: < 20 mm TL; Koumoundouros et al. 2009). The reason for not observing a similar trend in U_{crit} might be related to the fact that in critical swimming experiments, larvae are forced to swim constantly in a more unnatural way, as opposite to routine experiments.

Routine activity of red drum larvae has also been studied before (Fuiman et al. 1999, Smith et al. 2004), and swimming speeds are far better than speed recorded for gilthead seabream larvae. The measure of routine swimming speed, which increased significantly with ontogeny, included intervals of swimming and inactivity (Fuiman et al. 1999). The speed of recently hatched larvae (*ca*. 3 mm) averaged 1.2 mm s^{-1} and settlement stage larvae (*ca*. 25 mm) averaged 80.9 mm s^{-1} (Fuiman et al. 1999), which is much faster than the maximum speed recorded for gilthead seabream (**IV-A**).

In addition to critical and routine swimming speeds, the endurance of pre-settlement gilthead seabream larvae was assessed. The results (equivalent mean distance of 19.7 km) revealed higher values than those reported for settlement stage larvae of other

Sparidae: *Pagrus auratus*, 9.9 km, at a speed of 10 cm s^{-1} (Clark et al. 2005); *Sarpa salpa* and *Diplodus capensis*, 8 and 6 km, respectively, at a speed of 18 cm s^{-1} (Pattrick and Strydom 2009). These differences in performance might be related to the different speeds chosen for the endurance experiments, but can also be attributed to the ontogenetic stage itself. Nevertheless, these endurance abilities are considerably lower than the ones reported by Dudley et al. (2000) for three pre-settlement reef species of a temperate reef system, that swum at 13.5 cm^{-1}. Swimming times and associated distances varied among the species examined, but ranged from as high as 200 km (Scorpidae) down to 50 km (Mullidae and Monocanthidae). Once again, the range sizes of the tested species can explain the higher endurance abilities since Dudley et al. (2000) tested larvae with sizes up to 46 mm SL. Taken together, these results reinforce the need of comparing larvae of the same ontogenetic stages so that comparisons among species only reflect differences in swimming capabilities, and not an interaction between developmental stage and swimming performance.

2.2 Ontogeny of Swimming Speed – Temperate Gobiesociformes

The larvae of *Lepadogaster* spp. (**III-A**) were weaker swimmers when compared to the above mentioned species of temperate perciforms that have been studied and showed slower rates of speed increase with growth (0.5 to 1.6 cm s^{-1} per mm of growth for *L. purpurea*, and 0.3 to 1.2 cm s^{-1} per mm growth for *L. lepadogaster*) in contrast with gilthead seabream (0.4 to 4.2 cm s^{-1} per mm growth, **IV-A**), red drum (0.5 to 3.7 cm s^{-1} per mm growth, **III-B**), and other warm-temperate sciaenids and sparids (1.2 to 2.6 cm s^{-1} per mm growth, Clark et al. 2005). U_{crit} values ranged from 1 to 9.4 cm s^{-1} for *L. lepadogaster*, and from 1.2 to 6.5 cm s^{-1} for *L. purpurea*, over the common size range of 5.1 to 10.9 mm TL. Comparing each given size, *L. lepadogaster* swam faster than *L.*

purpurea and presented a faster swimming developmental rate, which may seem puzzling since these species are very closely-related, have similar morphologies and life-history trajectories, display comparable larval growth rates and size at hatching and occupy similar habitats on rocky reefs (Henriques et al. 2002). However, they differ with respect to the timing of the breeding season (*L. lepadogaster* spawns from March/April to June/July and *L. purpurea* from October/November to March/April) (Henriques et al. 2002). Their larvae are, therefore, exposed to different thermal environments: mean winter temperatures vary from 14.5-16 ºC while spring temperatures vary from 18-19.5 ºC (Henriques et al. 2002). In the present study, larvae of each species were tested at temperatures consistent with their natural environment, but temperature can affect swimming performance of fish larvae in two ways. First, fish muscle cells operate more efficiently at higher temperatures (Hunt von Herbing 2002). Second, temperature is necessarily linked to viscosity effects on fish larval motion (Podolsky 1994, Fuiman and Batty 1997, Hunt von Herbing 2002). The viscosity of sea water increases as temperature decreases, which means that either higher speed or greater size is required to reach a given Reynolds number (Re) at cooler temperatures (Fuiman and Batty 1997, Hunt von Herbing 2002). The Reynolds number (Re) is a measure of the ratio of forces that arise from viscous and inertial effects as a fish swims through water and it is commonly used to characterize different hydrodynamic conditions. It is calculated from the length and speed of a swimming organism and the kinematic viscosity of the surrounding fluid (Webb and Weihs 1986). This viscosity effect of temperature is greatest for smaller larvae and at lower water temperatures. At cooler temperatures, greater speeds are required to reach an inertial environment. Recently hatched larvae of both species were swimming in a viscous environment ($Re < 200$). During notochord flexion, larvae improved their swimming abilities, and after

notochord flexion was completed, individuals were able to swim at speeds that placed them outside the strictly viscous hydrodynamic environment (Re > 200). Nevertheless, *L. purpurea* had smaller Re numbers than *L. lepadogaster*, and this may account for differences found in critical speed between the two species. Although it would be meaningless to measure swimming performance at temperatures outside the range at which the larvae of a particular species are found in nature, it would be interesting to test both species at the same temperature, so that we could determine if differences in performance arise solely from differences in physical environment.

In both gobiesocids, critical speed increased steadily with size and age until settlement (*ca*. 10 mm); beyond this, they slowed down. The lack of swimming in larvae close to settlement is probably related to behavioural changes associated with the transition to a benthic lifestyle, rather than a decrease in the ability of larvae to swim. These individuals were completing metamorphosis and resembled newly settled fish, presenting the ventral adhesive disk of the adults.

In addition to critical swimming speed, routine speed was also determined for gobiesocid larvae (**III-A**). As seen for gilthead seabream larvae (**IV-A**), a non linear relationship between routine swimming speed and size was found. Despite a large variation of routine speed at any given size, an increase in swimming performance with development was observed in *L. lepadogaster*, until around 9 mm TL, after which it started to decrease. This decrease in routine swimming activity is coincident with the observed decrease in critical swimming performance and it might, again, be related to changes associated with a benthic lifestyle. Average routine speeds measured were low

(0.8 to 17.8 mm s^{-1} for *L. lepadogaster*, and 0.1 to 11.5 mm s^{-1} for *L. purpurea*), with larvae maintaining speeds of only *ca*. 18-19% of their U_{crit}.

Currently, there are no other studies on the swimming abilities of gobiesociforms and thereby, no direct comparisons within this order can be assessed.

2.3 Ontogeny of Swimming Speed – Temperate Pleuronectiformes

In chapter **IV-B** the critical speed of Senegalese sole, *Solea senegalensis*, was assessed from hatching until the beginning of metamorphosis. Critical swimming speed recorded for senegalese sole larvae in this study ranged from 0.4 to 5.0 cm s^{-1}. There are no data on critical speed of other Pleuronectiforms, but data available for routine speeds of temperate Soleidae, Pleuronectidae and Paralichthyidae report values of 0.5 to 3.0 cm s^{-1} (Blaxter 1986, Miller et al. 1988), which is close to what we found in this study (**IV-B**). These results also agree with the common perception that pleuronectiform larvae are poor swimmers. Opposite to what was found in other studies (Clark et al. 2005, Leis et al. 2006a, b, 2007, 2009a, b, chapters **III-A, B, IV-A**), the results found in **IV-B** show no significant relationship between critical speed and length. This may be attributed to the small size range of the tested larvae, or merely indicative of the poor swimming capacities of *Solea senegalensis* larvae. Nevertheless, a significant decrease on swimming abilities of post-flexion larvae was observed. This reduced swimming of larvae close to settlement is probably related to behavioural changes associated with a benthic lifestyle, since the post-flexion larvae were completing metamorphosis and resembled newly settled fish. This shift in swimming behaviour was also observed in gobiesocids (chapter **III-A**). Behavioural changes coupled with morphological changes are known to lead to variation in general metabolism (Bergeron 1982) and energy

expenditure during later development of flatfish, when their larvae are less active (Blaxter and Staines 1971).

2.4 Potential for Dispersal

The results so far discussed show that temperate reef fish larvae can have strong swimming capabilities. Whether these potential performances are actually reflecting those in the field remains yet to be determined, but they provide the potential for these larvae to strongly influence their dispersal. According to numerical models of circulation, both on temperate and tropical zones, only modest speeds (1 to 10 cm s^{-1}) are required to have large effects on dispersal (e.g. Pepin and Helbig 1997, Wolanski et al. 1997, Porch 1998). In this context, the studied species (**gobiesocidae, sparidae, sciaenidae and pleuronectidae**) were all capable of potentially influencing their dispersal range. Furthermore, a larva doesn't need to be an "effective swimmer" (i.e. be able to swim faster than ambient currents) to influence its dispersal (Leis 2006): horizontal swimming speeds less than 'effective speeds' can strongly influence dispersal trajectory if swimming direction is normal to the current direction. For example, in coastal waters, non-tidal currents are largely parallel to depth contours, so larvae over the continental shelf that are seeking to enter nursery grounds in shallow water nearshore or in estuaries, would only be required to swim at right angles to the flow (Leis 2006). Also, vertical positioning has long been recognized as having the potential to indirectly influence dispersal and even result in retention, as reviewed by Sponaugle et al. (2002). For example, larvae off the south-eastern USA continental shelf that moved inshore or were not advected, were found deeper in the water column than larvae that were exported from the shelf (Hare and Govoni 2005). These observations led Hare and Govoni (2005) to hypothesize that fish larvae can achieve onshore

transport by moving deeper in the water column in many marine systems. In another example, research on pleuronectiform fishes have concluded that because the larvae seem to be such poor swimmers, adjustment of vertical distribution is the prime behaviour used by flatfish larvae to achieve retention or transport to nursery areas (Bailey et al. 2005). This type of behaviour is probably adopted by Senegalese sole (**IV-B**) to avoid being flushed out of the nursery area, given the reduced swimming abilities throughout the entire pelagic stage.

Remaining near the bottom is another strategy used by larvae to avoid being swept out of coastal ecosystems (Sponaugle et al. 2002). A possible interaction between shallow depths, bottom complexity and the prevailing alongshore currents may create layers of different directions (Largier 2003); in these conditions, water flow is often slowed near the epibenthic boundary layer, increasing the potential retention of larvae close to the bottom (Breitburg et al. 1995). The height of this boundary layer varies depending on the current speed, the roughness of the bottom, density and type of vegetation that is growing on the bottom (Nowell and Jumars 1984, Folkard 2005). Larvae that occupy this epibenthic boundary layer will be subject to much reduced advection compared to larvae that remain above it (Black et al. 1991, Black and Moran 1991), and the typically measured or predicted water-column flow will not be relevant to their dispersal. Several studies show that fish larvae do indeed occupy the epibenthic boundary layer, at least for a portion of their pelagic period. In shallow (< 30 m) water off the southern California coast, net tows revealed that postflexion larvae of gobiids and two sciaenids left the middle portions of the water column, and were found almost exclusively in the epibenthic layer (Barnett et al. 1984, Jahn and Lavenberg 1986). In shallow water over oyster reefs in Chesapeake Bay, schooling goby larvae nearing settlement were

observed by divers over the bottom, and sheltering in the lee of objects (Breitburg 1991). In shallow inshore waters near rocky reefs in New Zealand, divers observed larvae of gobiesocids and tripterygiids in the benthic boundary layer (Kingsford and Choat 1989). In shallow inshore waters near reefs off southern England, larvae of several families (labrids, gadoids and ammodytids) were present in the epibenthic layer and around the reefs, but gobiid larvae were particularly abundant and seemed to occupy this environment throughout their larval stage (Potts and McGuigan 1986). In a temperate rocky reef (Arrábida Marine Park, Portugal), Beldade et al. (2006b) sampled the nearshore fish larval assemblages near rocky substrates, at three depths intervals, using a plankton net attached to an underwater scooter, and found higher larval abundances in deeper waters. Among the sampled species, two gobiesocids (*Lepadogaster lepadogaster* and *L. candolii*) were only present close to the substrate, being absent from surface and mid-water samples. It is very likely that gobiesocids are retained nearshore by occupying the epibenthic boundary layer. The considerable swimming abilities (**III-A**), together with the advanced stage of development at hatching (**II**) may favor larvae to swim close to the bottom soon in ontogeny, thus being retained.

In addition to these studies, fish larvae from several species, of mostly temperate locations, are known to occupy the benthic boundary layer (Marliave 1977, Leis et al. 1989, Steffe 1990, Kaufman et al. 1992). One central aspect for the interpretation of the patterns found in the aforementioned studies is the role of larval behaviour and its interaction with small-scale physical features of the nearshore environments at different geographic areas and oceanographic conditions. This is probably a fruitful direction for future studies of nearshore fish larval distributions.

2.5 Laboratory Limitations and the Use of Laboratory-Reared Larvae

Because the speed measurements that we used for studying swimming performance can only be made in an enclosed environment, these estimates of swimming behaviour may differ from those under natural conditions. One should then recognize laboratory limitations and be cautious when applying the observed results to larval dispersal models.

Laboratory studies have another disadvantage, as they tend to rely more on reared larvae. It is frequently assumed that swimming performance of reared larvae is inferior to that of wild larvae, although few direct comparisons have been made (e.g. Smith and Fuiman 2004). The use of reared larvae may not provide realistic results if their performance in the laboratory is different from that of wild larvae. Whenever possible, it is desirable to compare behaviour of reared larvae to wild ones (Leis 2006). On **chapter III-B** we have shown that reared and wild red drum larvae did not differ in swimming performance (measured as U_{crit}), suggesting that U_{crit} measured in lab-reared fish may accurately reflect natural swimming abilities in this species. These results indicate that research on reared larvae may provide naturalistic results for swimming performance. However, this can only be concluded for post-settlement larvae, since we did not catch wild larvae smaller than 8.25 mm TL. Smith and Fuiman (2004) found significant differences between reared and wild red drum larvae in routine speed at some developmental stages, but not all. For the other studied species (**chapter III-A**: *Lepadogaster lepadogaster*, *L. purpurea*, **chapters IV-A, B**: *Sparus aurata* and *Solea senegalensis*) efforts were made to collect wild larvae and test swimming performance in laboratory conditions. Attempts were, however, not successful and we hence need to be cautious when extrapolating data to field conditions.

Ideally, swimming speeds used to model larval dispersal should be based on measurements of larval fishes swimming in the sea, a measure called *in situ* speed (Leis et al. 1996, Leis and Carson-Ewart 1997). The greatest advantage of measuring swimming speed *in situ* is that, to some extent, it avoids the complications and ambiguities of laboratory measurements. The larva itself chooses the swimming speed. However, this method presents considerable challenges, particularly in temperate waters, where turbidity is usually high. Furthermore, not all species are amenable to it (Leis et al. 1996), as some species will sink to the bottom when released, some will simply float in midwater, some will attempt to settle on, or otherwise closely associate with the diver, and a few will simply hover in the water and closely watch the observer (Leis 2006). Another recent method for studying larval behaviour in the field was developed by Paris et al. (2008), Orientation with No Frame of Reference (OWNFOR). This system is deployed at sea and drifts while videotaping the movement of a larva placed within a clear, circular arena. This device allows not only measuring speed *in situ*, but also to detect and quantify the orientation of larval fish in the pelagic environment. Again, this system was first tested in coral reef waters, where turbidity is usually low, allowing perfect visibility of the traced larvae.

3. Life History Traits, Dispersal Potential and Implications for Population Connectivity

The spawning mode of fishes is among the principal life history trait that can influence their dispersal potential (Leis 1991, 2002, Cowen and Sponaugle 1997, Sponaugle and Cowen 1997, Leis and McCormick 2002, Sponaugle et al. 2002). Larvae hatching from demersal eggs tend to be larger and more developed than larvae of pelagic spawners (Thresher 1984), with better swimming performances, and may actively maintain their

positions nearshore (reviewed in Leis 1991). In contrast, larvae from pelagic spawners usually hatch smaller and have higher probability of being dispersed passively (Thresher 1984). On a size basis, there are no overall differences in speed between larvae from the two spawning modes. But on a post-hatch age basis, larvae hatching from demersal eggs are expected to initially have better swimming performance than larvae from pelagic eggs, and therefore, to have more influence on dispersal outcomes sooner in ontogeny. Several authors have found evidence of retention of reef fish larvae hatching from demersal eggs in temperate waters. On the Canadian west coast, larvae from demersal eggs, including Gobiesocidae, were dominant inshore and had a restricted alongshore distribution, favouring a rocky shoreline over sand (Marliave 1986). In contrast, larvae originating from pelagic eggs were more uniformly distributed both alongshore and offshore. At a similar fine scale in New Zealand, Kingsford and Choat (1989) also observed that larvae from demersal eggs were abundant near reef habitats of the adults, which was not observed for larvae from pelagic eggs. Gobiesocidae was one of the families being retained nearshore. Off SW Nova Scotia, larvae from demersal eggs dominated the inshore shallow-water environment, while densities of larvae originating from pelagic eggs were not correlated with bathymetry (Suthers and Frank 1991). The authors concluded that larvae from demersal eggs were more spatially persistent through the release of well-developed larvae from non-drifting eggs. In the Gulf of California, Mexico, several families, including sandy bottom and reef fish, utilized the near reef habitat throughout their development (Brogan 1994). These families, including Gobiesocidae, all spawned non-pelagic eggs and had well-developed hatchlings, but the larvae of other families, with similar spawning patterns, were not retained. At the Tsitsikamma National Park, South Africa, Tilney et al. (1996) found that gobiesocids and blennids were more abundant nearshore; from these, the

former were significantly more abundant over reefs than over sand. The fact that these larvae were present near reefs in different size classes seemed to indicate active larval retention nearshore. In tropical regions, larvae from demersal eggs are also found closer inshore than larvae from pelagic eggs (Leis and Miller 1976, Leis 1982). Leis (1982) concluded "that the most offshore pattern [predominantly larvae from pelagic eggs] was due to passive drift, while the inshore patterns [predominantly larvae from demersal eggs] were maintained by active swimming in conjunction with favourable currents". The author presented evidence of a tidal eddy and nearshore upwelling off the island of Ohau, Hawaii, as the putative mechanisms for inshore persistence of larvae derived from demersal eggs.

Despite the aforementioned evidence of nearshore retention of reef fish larvae, other studies do not support the hypothesis that reef fish larvae that hatch from non-pelagic eggs are retained mostly or exclusively near reefs on an exposed coast. Off central New South Wales, Australia, larvae from taxa with demersal eggs or that were viviparous were more abundant close to shore (Gray 1993). However, some larvae that originated from pelagic eggs also dominated nearshore. In northeastern New Zealand, the larvae from taxa that spawn demersal eggs were more abundant near reefs (Kingsford and Choat 1989). However, the pelagic-demersal distinction was not consistent, as the distribution of larval blenniids and monocanthids (demersal spawning) was not influenced by the proximity of reefs. Hickford and Shiel (2003) sampled ichthyoplankton on an exposed temperate coast and found that most of the taxa (the galaxiids and tripterygiids) that hatch from demersal eggs were also widely dispersed. Conversely, *Rhombosolea plebeian* (Pleuronectidae) which have pelagic eggs, were more abundant nearshore.

The presumable better swimming performance of larvae hatching from demersal eggs is not only advantageous for larval retention. It can also be an important survival advantage when larvae are dispersed and have to return to the reef to settle. The interaction of good swimming abilities with favourable oceanographic conditions may enhance recruitment success by facilitating transport back to the reef in the end of the pelagic period. For example, blenniids are demersal spawners and larvae hatch in an advanced stage of development (Faria et al. 2002, 2005, 2006), with the mouth and anus opened, pigmented eyes and almost no yolk, and well developed pectoral fins. This alone would suggest that blenniids are more predisposed to retention than dispersal. However, some blenniids have long pelagic durations (Ravéntos and Macpherson 2001, Faria et al. 2005, 2006), and therefore have a higher dispersal potential. Borges et al. (2007 a, b) found high abundances of recently hatched larvae of *P. pilicornis* close to shore and also offshore, in surface samples, but the more advanced stages of development were not found nearshore, which seems to indicate a dispersive pattern for this species. Olivar (1990) found *P. pilicornis* larvae over shelf waters of the Benguela region, being more abundant in the upper layers of the water column and suggested that this distribution could facilitate offshore transport associated to the coastal upwelling. Results from Brogan (1994) and Sabatés et al. (2003) also suggest dispersal of this family. In addition, in coral reef blennies, swimming speed can reach 26.4 cm s^{-1} (Leis and Carson-Ewart 1997). These characteristics, taken together, could favor survival in the pelagic environment and facilitate transport back to the reef in the end of the pelagic stage. Nevertheless, other studies have found evidence of retention in blennies (e.g. Tilney et al. 1996) which suggests that mechanisms of retention/dispersal are species specific and highly dependent on species behavioural abilities.

The gobiesocids studied in chapters **II** and **III-A** hatch from demersal eggs and present large larvae (> 5 mm TL) with functional eyes and developed fins and guts, and are able to swim actively immediately after hatching. Clingfish larvae have been found in several studies conducted in temperate nearshore rocky environments (Marliave 1986, Kingsford and Choat 1989, Brogan 1994, Tilney et al. 1996, Sabatés et al. 2003, Beldade et al. 2006a, Borges et al. 2007a, b). At the Arrábida Marine Park (Portugal), Beldade et al. (2006b) found that larvae of the genus *Lepadogaster* were mainly present near the bottom in all stages of development and R. Borges et al. (unpublished results), in the same geographic location, caught high numbers of *Lepadogaster* larvae of all size classes using light-traps placed near the bottom over these rocky reefs. This is indicative not just that these larvae seem to be growing and developing near the reefs, but also that they can actively behave and react to light very soon in development. Moreover, offshore sampling did not retrieve any larvae of these species (Borges et al. 2007b).

The more advanced stage of development at hatching (chapter **II-A**) and the known swimming capacity of these species (chapter **III-A**) might induce larvae to actively swim to the bottom, where flow is reduced (Breitburg 1991, Breitburg et al. 1995) and therefore avoid dispersal. Larvae that occupy this benthic boundary layer will be subjected to much reduced advection compared to larvae that remain above it (Black and Moran 1991, Black et al. 1991). Furthermore, clingfish larvae soon develop a ventral adhesive disk, which is an adaptation to their cryptobenthic mode of life, enabling them to counteract strong currents.

As opposed to gobiesocidae, the sciaenidae, sparidae and soleidae studied on chapters **III-B** and **IV-A**, **B** are all pelagic spawners, and recently hatched larvae of these species are very small (< 3 mm), with undeveloped eyes, guts and fins, and with virtually no swimming capacities. Until flexion was completed, swimming performance was limited

and after caudal fin formation, swimming performance improved at a species-specific rate (chapter 2 of the Discussion).

Red drum and Senegalese sole share a common life history trait being both estuarine dependent species. To enter an estuary, larvae may rely on large-scale displacement by major currents (Jenkins and Black 1994, Jenkins et al. 1997, 1999), or use selective tidal stream transport (Forward et al. 1999), which can be strongly dependent on vertical migration behaviour. Planktonic larvae of both red drum (Holt et al. 1989) and sole (Largardere et al. 1999) are known to be capable of migrating vertically in response to flood and ebb tides. Vertical distribution behaviour has long been recognized as having the potential to indirectly influence dispersal and even result in retention, as reviewed by Sponaugle et al. (2002).

Studies on pleuronectiform fishes, in particular, show evidence that because the larvae seem to be such poor swimmers, adjustment of vertical distribution is the prime behaviour used by flatfish larvae to achieve retention or transport to nursery areas (Bailey et al. 2005). The advantage of this strategy is that vertical currents tend to be weaker than horizontal currents and a relatively weak swimmer has a greater chance of regulating its transport through vertical movement than through horizontal swimming. Results from chapter **IV-B** seem to support the evidence that pleuronectiforms are poor swimmers and therefore may rely on other behaviours, such as vertical positioning, to avoid being flushed out of estuaries.

Red drum spawns in coastal waters near passes and inlets where the pelagic eggs and larvae are carried by tides and currents into shallow bays and estuaries (Holt et al.

1989). Larvae hatch with < 2 mm length (Holt et al. 1981). After tidal transport and initial settlement in habitats near inlets (at ca. 6 to 8 mm), juvenile red drum are thought to disperse throughout the estuary, with documented movements into shallow tidal creeks and lower salinity habitats (Peters and McMichael 1987, Stunz et al. 2002). Such dispersal can be passive (Jenkins et al. 1997, 1999) or may involve an active behavioural response (Leis et al. 1996, Stobutzki and Bellwood 1997). Results from chapter **III-B** indicate that red drum larvae have the swimming abilities necessary to respond rapidly to high current flows in order to remain in the settlement habitat, and/or migrate farther into the estuary. Moreover, a recent study on another temperate Sciaenidae (*Argyrosomus japonicus*) (Leis et al. 2006b) have shown, *in situ*, that more than 70% of the observed larvae had non-random trajectories. Swimming directionally is a prerequisite for horizontal swimming to be effective in influencing dispersal (Leis et al. 2006b).

The other pelagic spawner we studied, the gilthead seabream (chapter **IV-A**), is found in both marine and brackish water environments, such as coastal lagoons and estuarine areas in particular during the initial stages of its life cycle. Born in the sea during wintertime, the juveniles typically migrate in early spring towards protected coastal waters in search for abundant food and milder temperatures (trophic migration). At hatching, larvae of gilthead seabream are poorly developed, "little more than a yolk sac with a tail" (Leis et al. 2006), with very limited behavioural abilities, and most certainly subject to passive dispersal. However, results obtained so far suggest that with increased growth and development, larvae acquire swimming abilities that will allow for considerable movement, thereby potentially controlling their dispersal outcome. In addition to the strong swimming abilities, already confirmed in other temperate

Sparidae larvae (*in situ* studies: Trnski 2002, Leis et al. 2006b; laboratory studies: Clark et al. 2005, Pattrick and Strydom 2009), there are strong evidences that late stage Sparidae larvae swim in a directional (non-random) manner (Trnski 2002, Leis et al. 2006b).

Another important life history trait which has also been proposed as a measure of dispersal ability is the pelagic larval duration (PLD) (Sponaugle and Cowen 1997, Leis 2002, Mora and Sale 2002, Sponaugle et al. 2002, Lester and Ruttenberg 2005). In fact, at smaller spatial scales, the probability of self-recruitment is higher for species with a short PLD (Raventós and Macpherson 2001, Sponaugle et al. 2002), since the development is faster and a shorter pelagic phase increases the probability of the larvae staying close to their natal reefs. Some studies have demonstrated a positive relationship between PLD and distribution range in tropical reef fishes (Zapata and Herron 2002, Lester and Ruttenberg 2005). In contrast, other studies found no significant relationship between these two factors (e.g. Wellington and Victor 1989, Victor and Wellington 2000). For the studied species in this thesis (**chapters III and IV**), a positive relationship between PLD and dispersal range is likely to occur, as the next evidences show.

The available information for *Lepadogaster lepadogaster* shows that the pelagic larval duration varies between 11 and 18 days (Beldade et al. 2007, **chapter II**), which is a relatively short PLD for a temperate rocky reef species (Raventós and Macpherson 2001). This information, taken together with the other ecological and life history traits described previously (demersal eggs, large size at hatching, considerable larval development at hatching, strong swimming abilities, and presence in the benthic layer

and absence in more offshore waters), add to the growing body of evidence of nearshore retention of this species. Although no information is available for *L. purpurea*, laboratory rearing suggests that the PLD might be somewhat longer, close to 30 days (**chapters II and III-A**), which is considered an intermediate larval duration (Ravéntos and Macpherson 2001). We lack field data regarding spatial distribution of this winter-spawner gobiesocid, and thereby it is not possible to assume that this species is also retained nearshore.

Otolith-derived estimates of age indicated that red drum spends approximately 20 days in the plankton (Rooker et al. 1999). Although small and undeveloped (hatching size < 2 mm SL), red drum larvae are not dispersed far away. In the coastal region of Gulf of Mexico, for example, red drum eggs and recently hatched larvae are typically found within 20 km of the shore, inside the 18 meter-depth contour (Matlock 1990). Since swimming and sensory abilities only develop later in ontogeny, larvae may rely in other strategies to avoid being dispersed. Brown et al. (2005) have shown that physical processes can produce substantial retention of particles in the absence of behavior.

The Senegalese sole has, as well, a short pelagic larval duration. Metamorphosis occurs by day 14 after hatching, although the completion of eye migration and the change to a benthic habit occurs at approximately 18–20 days after hatching (Parra and Yúfera 2001). The presence of Senegalese sole larvae in ichthyoplankton surveys conducted in estuaries and adjacent coastal areas (Ramos et al. 2006, 2009, Vinagre et al. 2008) where spawning takes place, suggests that larvae may rely on retention strategies to avoid being flushed away from the nursery areas (Bailey et al. 2005). Estuarine circulation is influenced by tide, river flow, local wind, topography, among other

factors. Tides, in particular, are known to have a fundamental influence in larval transport in estuaries (Norcross and Shaw 1984, Boehlert and Mundy 1988). Selective tidal stream transport is widely accepted as the mechanism by which larvae move into estuaries (Jager 1999, Hare et al. 2005). Larvae are supposed to ascend vertically in the water column during the flood and return to the bottom when the tide turns, thus preventing being flushed back during ebb tide.

As opposite to the above mentioned species, the gilthead seabream has a long pelagic larval duration, prolonging their larval period over the 2 first months of life (Person-Le Ruyet and Verillaud 1980). To our knowledge, there are no studies on the dispersal of gilthead seabream larvae. However, data on other sparidae are available and may allow some inferences regarding dispersal range. In a temperate rocky reef (Arrábida Marine Park), Borges et al. (2007b) compared the horizontal spatial distribution of larval fish assemblages, sampling inshore (< 50 m from the reef) and offshore (2 miles). Sparidae (*Diplodus* spp., *Boops boops*, *Pagellus* spp., unidentified sparidae) was one of the most common family, and abundance was higher inshore than offshore. Most sparidae larvae were only caught in pre flexion stages, suggesting that this temperate reef functions as a nursery area for those species. The absence of more developed larval stages in the samples seems to indicate that these species disperse after hatching. However, a word of caution is needed here, since the authors only sampled with superficial trawls, and more developed larvae may occur in deeper waters. Supporting this evidence is the study by Borges et al. (2007a), who found larvae of the sparid *Boops boops* close to the reefs in all stages of development, which seems to indicate that this species is retained nearshore. *B. boops* is, within the sparids with known PLD, the species with the shorter pelagic duration and largest size at settlement (Ravéntos and Macpherson 2001), which

suggests that larval growth and development of behavioural abilities must be fast (Borges et al. 2007a). On another temperate rocky shore, in the northwest Mediterranean (Medes Islands Marine Reserve), Sabatés et al. (2003) also caught sparidae larvae (*Boops boops*, *Diplodus sargus*) in pre flexion stages very close to shore, but also over the continental shelf, at a considerable distance from the habitats of the adults of those species. This would suggest that these species are subject to significant dispersal during the planktonic stage.

The aforementioned studies suggest that the relationship between PLD and larval dispersal is species-specific, and most probably dependent on physical factors of the studied area. Wellington and Victor (1989) and Victor and Wellington (2000) have found a negative relationship between these variables, indicating that additional aspects of early life fish biology should be investigated (Armsworth et al. 2001, Shanks et al. 2003). PLD is taxon specific and influenced by environmental conditions such as temperature (Houde 1989, Pepin 1991, McCormick and Molony 1995). As a result, dispersal distances can be species, season, and location specific as well. Moreover, because many additional factors contribute to dispersal distance, simply setting dispersal distance solely as a direct function of PLD could be in many cases an oversimplification (Sponaugle et al. 2002).

Understanding the scale of dispersal during the pelagic larval stage is one of the major challenges facing marine biologists (Warner and Cowen 2002, Cowen 2002, Sale 2004, Leis 2006), as it is increasingly evident that management of marine populations, which has so often failed in the recent past, must take into account the scales over which these populations are demographically connected (Palumbi 2003, Cowen et al. 2003). The

levels of connectivity among local populations will determine whether they function as essentially isolated, ‘‘almost closed’’ populations, or as a metapopulation, with the separate dynamics of individual populations being buffered by subsidy of recruitment from other populations (Sale 2004).

It has become evident that, to be useful, predictions of dispersal must incorporate several things not normally taken into account (see Reviews by Cowen 2002, Sponaugle et al. 2002). Adult behaviour, specifically where and when spawning takes place, needs to be taken into consideration because it will determine where and when the propagules are put into the complex hydrographic systems surrounding reefs (Leis 2006). Hydrodynamics must be modelled (and ground-truthed) at multiple horizontal and vertical scales. In particular, micro scale circulation patterns in nearshore environments are of great importance since circulation patterns in these environments differ markedly from the ocean environment. Some nearshore environments have particular oceanographic features that can facilitate larval retention (Harris et al. 1999, Pineda 2000, Sponaugle et al. 2002, Largier 2003) and these need further investigation.

Larval-fish behaviour must be added in the form of vertical distribution, swimming speed and endurance, sensory perception, and when in ontogeny (and how) their development can affect dispersal (Leis 2006). Finally, larval-fish behaviour is flexible and is expected to differ depending on location, orientation and on the presence or absence of predators (Leis 2006). Recent models of dispersal attempt to incorporate some of these things, including larval-fish behaviour (Wolanski and Sarenski 1997, Wolanski et al. 1997, Porch 1998, Armsworth et al. 2001, Lindeman et al. 2001), but there is a long way to go to fully understand dispersal. In this context, behavioural data

obtained in **chapters III** and **IV** are of great relevance for inclusion in future models to be developed on the Portuguese nearshore reefs.

4. Temperate Reef Fish Larvae *vs*. Tropical Reef Fish Larvae

Cowen and Sponaugle (1997) point out that research on temperate fish larvae have focused on feeding and predation (mortality), whereas studies of coral reef fishes has emphasized the retention/dispersal question. Without denying the need of more studies on the sources of mortality, more work on retention/dispersal is in great need, in particular in temperate nearshore systems. Processes that control population dynamics of temperate rocky reef fish can differ from those affecting coral-reef fish populations (Ebeling and Hixon 1991) and, consequently, extrapolations from results of rocky shores or coral-reef habitats must be tested. Nevertheless, given the similarities in ecology and some life history traits between larvae of coral and temperate rocky reefs, it is highly probable that these populations share similar regulation mechanisms, such as retention.

As is can be seen in Table I (Appendix 1), the great amount of studies on larval swimming abilities have been conducted on tropical perciform species, and have concentrated mainly on critical and endurance speed, and more recently, on *in situ* speed. Tropical fish larvae generally appear to be much better swimmers than temperate fish larvae when short-term and prolonged activity are compared. Settlement-stage reef fish can swim several km's and their critical swimming speed reach 100.8 cm s^{-1} (see Table I). As opposite, the few studies on swimming behaviour of temperate fish larvae have concentrated on a few pleuronectiform and clupeiform species, and have focused on measures of routine speed, with speeds ranging from 0.5 to 5 cm s^{-1} (see Review by Blaxter 1986; see Table I) when swimming freely in aquaria. However, recent research

on temperate perciformes is increasing fast, and the few existent studies focusing on the swimming abilities of a wider range of temperate species belonging to Mullidae, Monocanthidae, Scorpidae (Dudley et al. 2000), Percichthyidae, Sciaenidae and Sparidae families (Trnski 2002, Clark et al. 2005, Leis et al. 2006b, Pattrick and Strydom 2009, **chapters III-B**, **IV-A**), have shown that these larvae tend to have swimming abilities comparable to those of tropical species. Nevertheless, the taxonomic base upon which our understanding of larval fish behaviour is based is still a small contribution given the high diversity of fishes (about 24,000 teleost species of which about 60% are marine; Nelson 1994).

Another important variable that can be seen in Table I is the range size of tested larvae. Most studies, particularly on tropical species, have concentrated on late stage larvae, close to settlement. However, in order to fully access the influence of swimming behaviour in dispersal it is important to know when, during ontogeny, these abilities develop. From Table I analyses it is possible to see that the most recent studies begin to take ontogeny in consideration. Moreover, a large amount of comparisons of swimming performances are frequently made regardless of developmental stage, but is evident that performance of pre-settlement stage larvae is not comparable to performance of younger stages. In this sense, size, or ontogenetic stage, needs to be taken into account in future comparisons.

Other than taxonomy and differences in developmental rate, temperature is probably the most relevant confounding factor for many of the comparisons among tropical and temperate species. Temperate marine fish show large responses to temperature change through swimming performance at all stages of development. Swimming temperature is

known to affect critical swimming speed in larvae (e.g. Guan et al. 2008) and spontaneous swimming activity in juveniles (e.g. Fuiman and Ottey 1993). Temperature-induced changes in rates of larval morphological development may also have indirect effects on swimming performance, because the development of key functional structures such as muscles, gills and biochemical pathways are retarded or accelerated (Taylor et al. 1997). Moreover, temperature is also necessarily linked to viscosity effects on fish larval motion because larvae are small and slowly moving in aquatic systems (Podolsky and Emlet 1993, Fuiman and Batty 1997, Hunt von Herbing 2002); the effects are especially significant for small larvae in cold water (Gillis 2003) before they become sufficiently large and fast that swimming motion occurs in an inertial rather than viscous environment.

Reynolds number was calculated for critical swimming experiments of the studied species (**Gobiesocidae, Sciaenidae, Sparidae, Soleidae**) to determine whether larvae were swimming at conditions that where either viscous (conventionally considered being Re < 200) or inertial (Re > 200), depending on speed. The threshold of 200 is the most conventionally used number (Webb and Weihs 1986), and although recent experiments indicate that the viscous environment could extend to values of Re = 300, and a fully inertial environment may not come into play until *Re* > 1000 (e.g. Fuiman and Batty 1997), these thresholds are also species-specific and since we lack information for most of the studied species (except red drum) we chose to use the most conventional and conservative value of Re. In the case of red drum, the thresholds are known: viscous forces dominate when Re < 600 (Fuiman and Batty 1997, Sarkisian 2005) and move to inertia-dominated forces when Re > 1300 (Sarkisian 2005). With the exception of senegalese sole, our results show that after notochord flexion

(*Lepadogaster lepadogaster* and *L. purpurea*: 8-9 mm length [**II-A**, **III-A**]; *Sparus aurata*: 6-7 mm length [**IV-A**], *Sciaenops ocellatus*: 6-7 mm length [**III-B**]) the larvae of the species we studied were able to swim at speeds that placed them outside the viscous hydrodynamic environment. Other studies on the ontogeny of swimming performance, whether on temperate (e.g. Clark et al. 2005), or tropical (e.g. Leis et al. 2007) species, have also observed that after notochord flexion, larvae were clearly capable of swimming in an inertial environment.

Leis (2006) performs a detailed analysis on the relationship between speed and length in seawater showing the combination of speed and size at which Reynolds numbers of different magnitudes are achieved. For a larva of a fixed size, greater speeds are required to achieve any given Re value in cooler water. The effect is greater for smaller larvae and in cooler temperatures. For temperatures above 20º C fish larvae swimming at critical speed will reach an inertial hydrodynamic environment by 5 to 8 mm, depending on species (Leis 2006). Therefore, for most of the pelagic larval phase, larvae in warmer water will be capable of swimming in a largely inertial hydrodynamic environment. In contrast, because of their slower speed, and the increased viscosity of cold water, larvae of cool temperate species will not reach the inertial environment until 10 to 11 mm (Leis 2006).

In conclusion, temperature may have a fundamental role in determining larval fish swimming abilities and, once again, caution is needed when making comparisons without taking into account temperature differences. If temperature does have a substantial effect on swimming performance and/or larval activity levels, this suggests that there large differences in the fundamental mechanistic processes behind larval

growth and survival at different latitudes may exist (Fisher and Leis 2009). Furthermore, considering the influence that temperature has on growth and developmental rates, it can be expected that larvae from tropical regions may increase swimming capabilities at a much faster rate than temperate larvae, having a greater influence on their dispersal patterns (Fisher and Leis 2009). Nevertheless, the number of studies that allow separating taxonomy from temperature effects are still insufficient.

At a larger scale, differences in dispersal patterns will have consequences to the design of marine protected areas (MPAs) both in tropical and temperate regions. MPAs are found with greater frequency at lower latitudes, and consequently, most research on their design, implementation, and evaluation is based on tropical examples. Laurel and Bradbury (2006) hypothesized that two patterns in dispersal would emerge from environmentally associated changes with latitude, due to an extended pelagic larval duration (PLD) at higher latitudes. The authors concluded that marine fish populations seem to be less isolated and experiencing greater levels of gene flow at higher latitudes, being very likely the result of an increased PLD, that, in turn, translates into larval dispersal over greater geographic scales, and their data suggest that spatial management strategies such as implementation of marine reserves will be less effective at high latitudes if they are constructed at scales similar to reserves in tropical regions. Some recent reviews (e.g. Bradbury et al. 2008) also report a significant increase in PLD with latitude using several groups of marine fish species. However, the association of longer pelagic durations with higher latitudes may not always be the case. Contrary to what is many times assumed, Borges et al. (2010) found that temperate gobies presented a larger size at settlement but a shorter PLD than tropical gobies. These authors call attention to the fact that the presumed differences between temperate and tropical

systems may be smaller than what is normally assumed when the same taxonomic group is compared and the species' life history characteristics are considered.

5. Influence of Condition on Swimming Performance

Larval condition and growth are the main determinants of the development of larval swimming abilities (Mora and Sale 2002). Fast growth is frequently associated with high condition; moreover, high condition larvae can be larger and heavier, with greater concentrations of lipids, carbohydrates and proteins, greater capacity for energy storage, and increased musculature development compared with low condition larvae (McCormick and Molony 1993, Green and McCormick 1999). Variable condition can then lead to differences in behavior, such as swimming and foraging (Stobutzki 1997, Green and McCormick 1999, Sogard and Olla 2002), among individuals. Swimming capability associated with higher condition can result in increased response or quicker evasion during predator attacks (Chick and Van den Avyle 2000, Grorud-Colvert and Sponaugle 2006). Obviously, the outcome of this interaction has direct relevance for larval survival.

The influence of starvation on condition and swimming behaviour of pre-metamorphosis larvae of two important commercial species, gilthead seabream and Senegalese sole, was investigated in **chapters IV-A, B**. Responses of both species were quite similar when analyzing the effects of a long-term starvation period (3 days for gilthead seabream and up to 4 days for Senegalese sole) on critical swimming speed (the common measure of swimming performance in both species). U_{crit} of starved larvae was not significantly different from U_{crit} of fed larvae after 3 to 4 days of food deprivation. The effect of starvation on routine swimming speed was also investigated

on gilthead seabream larvae, and results are in accordance to what was found for U_{crit} experiments: 3 days of starvation were not enough to influence routine behaviour of pre-metamorphosis larvae. The lack of food deprivation effects on short term swimming behaviours, such as U_{crit} and routine speed, has been reported in other studies. Laurence (1972) studied sustained swimming abilities and activity level of fed and starved largemouth bass (*Micropterus salmoides*) larvae at 19 ºC, and found that differences in swimming activity were only notable after a period of 4 days of starvation. Similarly, Yin and Blaxter (1987) reported decreased responsiveness and escape speed for starved herring, cod and flounder, reared at 9-10 ºC, but the effects of starvation were not evident until larvae had starved for several days. Chick and Van den Avyle (2000) observed a similar pattern when examining the effects of feeding ration on the routine swimming speed of larval striped bass (*Morone saxatilis*). More recently, Skajaa and Brown (2007) concluded that escape responses in food-deprived cod (*Gadus morhua*) larvae were in general not affected by 3 days of food deprivation at 10 ºC. On the contrary, Grorud-Colvert and Sponaugle (2006) found that bluehead wrasse *Thalassoma bifasciatum* recruits fed for 1 wk at higher levels grew faster and swam faster than food-deprived recruits. High condition recruits also evaded a simulated predator threat at faster speeds than the low condition recruits and exhibited less risk-taking behavior by sheltering more in the presence of a predator threat and consuming less food. However, results from Grorud-Colvert and Sponaugle (2006) cannot be directly comparable to ours (**chapters IV-A, B**) and to the above mentioned studies since the authors were testing newly settled recruits, as opposite to larvae; moreover, the starvation period used by Grorud-Colvert and Sponaugle lasted for longer (1 week) than employed in the other studies.

Although critical and routine swimming behaviours were not affected by food deprivation, the endurance abilities of gilthead seabream larvae were significantly affected by starvation. This is not completely surprising, because during endurance swimming larvae use all their energetic reserves, namely the lipids, carbohydrates and proteins (Stobutzki 1997). Larvae that were fed *ad libitum* prior the experiment had clearly more energetic reserves than the ones that were starved, suggesting that reserves of unfed larvae were depleted faster than reserves of fed larvae. However, these results demonstrate that larvae that do not show differences in critical and routine speeds, may have very different capacities of long-distance swimming.

Altogether, the results of a variety of studies suggest that even deprived of food, larvae may be capable of performing escape and foraging behaviours (activities in which critical and routine speeds might be involved), but sub-lethal effects of starvation may still affect dispersal potential by greatly reducing endurance swimming, therefore compromising subsequent survival and recruitment to the adult population.

With respects to growth (in length), gilthead seabream and Senegalese sole exhibited different responses to starvation. Growth of starving gilthead seabream was not significantly different from growth of fed larvae. In contrast, starvation had a significantly negative influence on growth of Senegalese sole larvae. Reduced growth rate caused by starvation will extend the duration of vulnerable ontogenetic stages and, as there are more potential predators for small larvae, starved larvae might suffer a higher cumulative rate of mortality (Cushing 1975, Shepherd and Cushing 1980). It is possible that differences in growth response to starvation between the two species might be related to the way larvae use their energy content. Parra and Yúfera (2001) studied

growth, energy content, and energy efficiencies in larvae of these two species and concluded that, during the first month after hatching, *Solea senegalensis* invested more energy on growth than on metabolic processes, as opposite to *Sparus aurata*. Therefore, in a situation of food deprivation, growth of Senegalese sole larvae will be affected more rapidly than growth of gilthead seabream.

One of the advances of the studies described on **chapters IV-A and B** is the fact that we not only correlated starvation with swimming performance, but we also measured condition, using morphometric (Fulton's *K*) and biochemical (RNA/DNA ratio) indices. Fulton's *K* is a commonly used indicator of an individual's general well-being (e.g. McComirck and Molony 1993, Booth and Hixon 1999, Hoey and McCormick 2004, Grorud-Colvert and Sponaugle 2006, Holmes and McCormick 2009) and is based on the assumption that heavier fish for a given length are in better condition (Ricker 1975, Suthers 1998, Grant and Brown 1999). It was measured for gilthead seabream larvae (**IV-A**) and results showed that fed larvae tested in the endurance trials had a higher post-trial Fulton's *K* when compared to starved larvae, suggesting that unfed larvae had either grown slower (in terms of weight) or had lost more body mass while being swum. Fulton's *K* was not affected by feeding treatment in the critical speed and routine swimming trials, which is probably related to the fact that these swimming types are short-term measures of performance, and weight itself is not affected during the trial. These results agree with the aforementioned results on the effect of starvation on swimming performance of gilthead seabream larvae. As already discussed, endurance was the only type of swimming affected by starvation, most probably due to substantially larger energy reserves needed to fuel this type of swimming.

RNA/DNA ratio has been shown to respond to changes in feeding conditions and growth in periods as short as one to three days in a variety of fish species and is a reliable growth rate estimator which has been applied in numerous field assessments (Rooker and Holt 1996, Buckley et al. 1999, Gwak and Tanaka 2001, Chícharo et al. 2003). In both studies, RNA/DNA ratio response to starvation showed some sort of latency. In gilthead seabream, changes in this ratio were only detectable after a 3 day period of starvation, but in Senegalese sole, RNA/DNA ratio was not affected by a 4 day period of food deprivation. This type of latency has been demonstrated in other fish species. Clemmesen (1994) reported that fed and starved herring (*Clupea harengus*) larvae (>10 days) could be distinguished only after 3 to 4 days. Similarly, Rooker and Holt (1996) reported that fed and starved larval and juvenile red drum (*Sciaenops ocellatus*) could be discriminated within 1 to 2 d of food deprivation using the same ratio. The fact that a period of 4 days of starvation did not have consequences on RNA/DNA ratio of sole larvae and a period of 3 days of starvation induced changes on the same biochemical ratio on gilthead seabream larvae, might be related to the known fact that flatfish larvae are more resistant to starvation (Clemmesen 1987, Yin and Blaxter 1987). When compared to other species with small eggs and yolk-sac larvae, such as *Engraulis mordax* (Lasker et al. 1970), *Paralichtys californicus* or *Hypsopsetta guttulata* (Gadomski and Petersen 1988), and to species carrying larger yolk reserves, such as *Clupea harengus* (Blaxter and Hempel 1963) or *Ammodytes americanus* (Buckley et al. 1984), flatfish larvae seem better adapted to low food conditions.

Richard et al. (1991) studied the effect of starvation on RNA/DNA ratio in *Solea solea* larvae and concluded that the timing of the beginning of starvation was an important factor in determining larval condition. Larvae starved from the beginning of exogenous

feeding showed a sharp decrease in the RNA/DNA ratio, and could be distinguished from fed larvae after 2 or 3 days of starvation. However, for larvae starved after days 5 and 10, the food deprivation interval must be longer to show a clear effect in condition, and in juveniles starved after Day 14 (after metamorphosis), the RNA/DNA ratio was not significantly different from that for juveniles fed throughout the experiment (Richard et al. 1991).

6. Comments on Study Methods and the Need for Standardization

It is now clear that comparisons of swimming speed among taxa should take into account phylogeny, developmental state and temperature. However, there is another aspect that needs to be critically analyzed: the variety of methodologies used to measure swimming abilities. These can be important sources of variation, potentially biasing comparative studies of swimming performance, specifically for intraspecific comparisons (Kolok 1999). Given the variety of available methods, it is important to define a series of guidelines for standardization, in order to allow more meaningful comparisons among taxa in the future. The adoption of widely used protocols and the improvement of others are herein proposed.

Numerous studies have used U_{crit} to examine the swimming abilities of fishes in relation to environmental factors (e.g. Green and Fisher 2004), pollutants (e.g. Kennedy et al. 1995), and growth rate (e.g. Kolok and Oris 1995). Because U_{crit} can be correlated to other swimming measures, such as *in situ* swimming (Leis and Fisher 2006) and sustainable swimming speeds (Fisher and Wilson 2004) it can be used to estimate the effectiveness of a species' swimming abilities. The U_{crit} method is also useful because the experiments are simple and can be replicated easily allowing for a comparison of

swimming abilities from a breadth of taxonomic groups and geographic regions. However, although many of these studies cite Brett (1964) as the source of the methodology, most have modified the original technique in some way. As a result, there exists no standard protocol in the literature for conducting U_{crit} experiments (Kolok 1999) and aspects as velocity increment size, interval length, and the number of fish per channel varies among studies. In an attempt to test these variables, Hogan et al. (2007) measured the critical swimming abilities of late-stage larvae of 46 species from 21 families of marine fishes and tested the robustness of the U_{crit} technique to variations in methodology. The authors concluded that U_{crit} is relatively robust to variations in methodology (i.e., varying the length of the time interval and varying the number of fish in the chamber). Although a standard protocol for measuring U_{crit} has never been proposed, the protocol and swimming chamber developed by Stobutzki and Bellwood (1994, 1997), who first studied swimming abilities of late-stage coral reef fish larvae, has been widely adopted by larval ecologists. In this sense, this protocol should be adopted worldwide, given the efficiency and robustness of the technique to slight variations in methodology.

Endurance speed is one of the most common measures used to assess the swimming potential of larvae, and it has been shown that late stage larval fishes are capable of swimming for long periods of time (days) and cover long distances (100's of kilometers, in some cases). In this method, larvae are forced to swim at a given speed until exhaustion. However, endurance ability varies substantially with the chosen swimming speed (Fisher and Bellwood 2002, Fisher and Wilson 2004). Prior studies of reef fish larvae have used an experimental speed of 13.5 cm s^{-1} (Stobutzki and Bellwood 1997, Stobutzki 1997, Dudley et al. 2000), selected to represent the average current

speed around reefs in the Lizard Island region. However, ocean current speeds vary both spatially and temporally. Furthermore, swimming times and distances can be greatly increased in the presence of food (Fisher and Bellwood 2001, Leis and Clark 2005).

Larvae will have the greatest impact on their dispersal if they swim at the fastest speed they can sustain without exhibiting the effects of exhaustion. This will maximize the total distance covered in a given time. Studies examining the sustained swimming capabilities of unfed larvae conducted over time frames longer than 24 h may simply be measuring energy reserves of larvae, rather than sustained swimming abilities. For this reason, we herein suggest that an appropriate time scale for measuring sustainable swimming speed will be of 24 h, as Fisher and Wilson (2004) propose. This time frame is also relevant when considering the ability of larvae to influence their dispersal patterns because in many locations, ocean currents are tidally dominated. For determining the appropriate speed, larvae should be swum at intervals of speed decreasing by 5 cm s^{-1} until the speed at which 90% of the larvae can swim for 24 h is determined.

Probably, the most problematic measure of swimming speed is routine speed measurement, given the enormous variety of methodologies and the series of variables capable of influencing routine activity. Factors such as: tank size and volume, food distribution, density of larvae, starvation, light conditions, time of the day, turbulence, variability in individual behaviour, among others, influence swimming speed of larvae, making almost impossible to compare routine speeds among taxa. Herein it is proposed that measures of routine speed are conducted on the rearing tank, without turbulence or food, and repeated at different times of the day, including at night, since it is clear that some larvae may be more active at night, especially settlement-stage larvae (Fisher and

Bellwood 2003). Despite the suggested guidelines, measures of undisturbed swimming speeds will most probably be conservative compared to what may be expected to occur in the field, given the amount of variables that affect routine behaviour and that are impossible to reproduce in laboratory.

An alternative to the laboratory study presented here is the work of Leis and Carson-Ewart (1997), which examines the swimming speeds of larvae *in situ*. The advantage of their method is that the behaviour of larvae can be examined in the presence of natural sensory cues, natural levels of turbulence and water flow, as well as with the full range of horizontal and vertical spatial scales. The problem with *in situ* measures of swimming speed, however, is that it is impossible to remove the effects of diver presence from observations of swimming speed and behaviour. In addition, such measurements can only be made for older-stage larvae that are large enough to be followed on SCUBA, and are further restricted to those species and stages that are available from light traps. More recently, a new device for studying larval behaviour in the field was developed by Paris et al. (2008): Orientation with No Frame of Reference (OWNFOR). This system is deployed at sea and drifts while videotaping the movement of a larva placed within a clear, circular arena. This device allows not only measuring speed *in situ*, but also to detect and quantify the orientation of larval fish in the pelagic environment. Again, this system was first tested in coral reef waters, where turbidity is usually low, allowing perfect visibility of the traced larvae. Nevertheless, these two methods for studying larval behaviour *in situ* have proven to be the best available methods for studying what larvae are really doing in their natural environment, and efforts should be made to adopt these methods whenever possible.

7. Final Remarks

Overall, the present thesis adds more results to the growing body of evidence that fish larvae are far from simple, uninteresting, passive particles as has been put forward by the traditional view of larval biology. Upon hatching, larvae of most studied fish species have limited swimming abilities, but by the time flexion is completed and the caudal fin formed, larvae swimming at critical speeds were in an inertial environment and higher swimming speeds could be attained. Furthermore, larvae hatching from demersal eggs, such as gobiesocids, were capable of swimming against a minimum flow of 1 cm s^{-1} immediately after hatching. As opposite, larvae hatching from pelagic eggs only developed their swimming abilities later in ontogeny, indicating that in fact larvae from different types of spawning can have different behavioural capabilities. Results from swimming performance of Senegalese sole confirm the view that pleuronectiforms are poor swimmers. This estuarine-dependent species may rely on other behavioural mechanisms to avoid being flushed out of the nursery area. On the other hand, swimming performance of the studied temperate perciform species questions the view that temperate species are worse performers than tropical fish larvae. In fact, results suggest that there isn't a strong difference in swimming speeds between temperate and tropical fish larvae when taxonomy, morphology and developmental stage are taken in consideration.

Studies on the influence of larval condition on swimming performance suggest that even deprived of food, larvae may be able of performing escaping and foraging behaviours (activities in which critical and routine speeds might be involved), but sub-lethal effects of starvation may affect dispersal potential by greatly reducing endurance

swimming, and therefore compromise subsequent survival and recruitment to the adult population.

I believe the results presented herein may lead the way for future studies on the influence of larval behaviour on dispersal. The data on swimming performance of these species can be taken into account in numerical and biophysical dispersal models and other applications that require information on the development of swimming ability in fish larvae, as well as on the interpretation of ecological data derived from plankton samples in coastal environments.

8. Future Research Directions

The present work represents a significant contribution to the implications of larval behaviour in temperate ecosystems. However, the range of tested species is narrow and future work must include a wider representation of the great taxonomic diversity found on temperate reefs. Since most investigations have concentrated on late-stage larvae, rarely incorporating the ontogeny of behaviour, I believe the approach followed on the present work should be widely adopted. The lack of knowledge about the ontogeny of behaviour in larval fishes inhibits the development of realistic dispersal models. Therefore, future studies will need to determine when, during ontogeny, larvae are sufficiently developed to begin to control their trajectory and how do larval behaviour influence their positioning in the water column. This will require studies on laboratory-reared larvae, but attempts should be made to ensure that results on lab-reared larvae are representative of capabilities and performance of wild larvae. In this sense, efforts should be made to use wild-caught larvae whenever possible.

Perhaps one of the greatest challenges facing the majority of marine reef organisms with larval stages that potentially disperse and develop in offshore waters, is how to locate the relatively rare patches of coral-reef and other rocky nearshore settlement habitats. The answer must rely partly in the sensory modalities of fishes since larval recognition of settlement habitat can be based on the detection of conspecifics and/or characteristics of habitat using visual (e.g. shape of the hard substrate), chemical (e.g. odour of organisms) and/or mechanical (e.g. biological acoustic sounds) cues. The great gap in our understanding of the sensory capabilities of reef fish larvae must be filled and future research on temperate ecosystems must give this matter top priority.

It will also be of great interest to integrate larval behaviour with biophysical models of larval dispersal, as this integration should provide much insight into the realities of dispersal and retention. This is critically important to our understanding of population connectivity and to management decisions, including the design of MPA networks. Larger MPAs will be required to promote recruitment benefits within boundaries when dispersal is large; on the other extreme, populations that are little connected have reduced ability to recover from local depletion or habitat degradation and should have conservation priority (Roberts et al. 2006, Jones et al 2007).

There is, as well, a need to relate performance with variability in early life history, as environmental variability can strongly affect traits such as larval growth, the onset of relevant ontogenetic events or pelagic larval duration. In this context, otolith microstructure is a major tool for studying the ecology of fish larvae and juveniles. Use of the wealth of data contained in the microstructure of fish otoliths in one of the most significant advantages that studies of fish populations have over similar studies of

invertebrates. From the otolith microstructure, valuable information on early life history traits, such as age, planktonic larval duration, origin, growth rates and environmental history, can be obtained (see Sponaugle 2009). Understanding how larval past history influences performance, condition, and fate of successful settlers is critical for management, because it is the fitness of survivors that will directly determine survival and future recruitment. In this sense, the use of otolith microstructure analysis to further understand variability of early life traits, larval condition, and how this variability influences performance and survival is a major tool to be used in the future.

In the pelagic environment the energetic cost of swimming may determine the extent to which different taxa modify their dispersal by directional swimming. This energetic cost may also influence post-settlement survival and growth on the reef. Therefore, given the potentially large metabolic cost of swimming and the long distances swum by the late pelagic stages of fish, the question arises: how effective is larval physiology supporting swimming; and what effect do these swimming episodes have on their energy reserves? A recent study has revealed surprising results of metabolic efficiency by coral reef fish larvae (Nilsson et al. 2007) which showed that pelagic larvae were extremely efficient in O_2 uptakes, and this rate decreased after settlement, when fish must adapt to a more hypoxic environment. Despite the impressive results, there are no available studies for temperate reef fish larvae and future studies should point in this direction.

Taxonomic work on the pelagic stages of reef fishes must not be set in second place. Inability to identify larvae to the species level, particularly early stages, is a major impediment to the understanding of distribution patterns, feeding ecology, condition and many other issues. Therefore, studies on larval descriptions will always be a

fundamental research area in larval fish ecology. Finally, the use of genetic markers is proving to be a powerful tool for the correct identification of larvae collected in the plankton and thereby a comprehensive use of this tool will help resolving many of the identification problems and doubts that still persist.

Finally, future comparisons of swimming speed among taxa should take into account phylogeny, methodology, developmental state and temperature. Most recent studies have begun to take these factors into consideration when making interspecific comparisons and, as a result, the extent to which tropical larvae might swim better than temperate larvae is now unclear.

9. References

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Appendix 1

TABLE I. Compiled information of critical, routine, endurance and *in situ speed* of tropical and temperate marine fish larvae and associated size range and temperature. Values are drawn primarily from reviews: (1) Blaxter 1986; (2) Bailey et al. 2005; but with additional values from the primary literature: (3) Wuenschel and Able 2008; (4) Fisher et al. 2005; (5) Hunter 1972; (6) Guan et al. 2008; (7) Leis et al. 2007; (8) Leis and Carson-Ewart 2001; (9) Leis and Fisher 2006; (10) Dudley et al. 2000; (11) Leis et al. 2009a; (12) Trnski 2002; (13) Leis et al. 2009b; (14) Clark et al. 2005; (15) Chick and van Den Avyle 2000; (16) Peterson and Harmon 2001; (17) Fisher et al. 2000; (18) Leis and Clark 2005; (19) Leis et al. 2006a; (20) Fuiman et al. 1999; (21) Fukuhara 1987; (22) Pattrick and Strydom 2009; (23) Koumoundouros et al. 2009; (24) Ryland 1963; (25) Fisher et al. 2007; (26) Fisher and Bellwood 2003; (27) Leis et al. 2006b (28) Fukuhara 1987; (29) Doi et al. 1998; (30) Westernhagen and Rosenthal 1979; (31) Hunter and Kimbrell 1980. Region: TROP- tropical; TEMP- temperate (including subtropical). Some studies lack information on larval size, and report only the ontogenetic stage (hatching and/or settlement). Other studies only give information on mean speed and mean size, and the range is not provided. See References on chapter 9 of General Discussion.

Order	Family	Species	Region	T (ºC)	Size range (mm)	Swimming measure				Reference
						Ucrit	Routine	Endurance	*In situ*	
Anguilliformes	Anguillidae	*Anguilla rostrata*	TEMP	4-21	48.7-68.1	6.5-21.1				3
	Congridae	*Conger oceanicus*	TEMP	14-24.5	68.3-117.8	4.1-26.8				3
Cluperiformes	Clupeidae	*Clupea harengus*	TEMP	9.5	7.4-12.5		10-23			30
		Jenkinsia spp.	TROP	28-30	37.7	23.4-				4
		Spratelloides	TROP	28-30	30.8	4.7-28.9				4
	Engraulidae	*Engraulis mordax*	TEMP	17.5	4-25		1-20			5
Gadiformes	Gadidae	*Gadus morhua*	TEMP	6-10	0.5-1.42	1.2-9.7				6
Gobiesociformes	Gobiesocidae	*Lepadogaster lepadogaster*	TEMP	16.4-17.5	5.1-10.4	1-9.4	0.8-17.8			**Chapter III-A**
		Lepadogaster purpurea	TEMP	14.9-15.4	5.3-10.9	1.2-6.5	0.1-11.5			**Chapter III-A**
Gonorynchiformes	Chanidae	*Chanos chanos*	TROP	25	6-13	1-23				7
Perciformes	Acanthuridae	*Acanthurus triostegus*	TROP		Settlement				8.7-65.3	8, 9
		Acanthurus sp	TROP		Settlement	61.5			24.7	9
		Acanthurus sp	TROP	28-30	26.9	58-9-				4
		Acanthurus bahianus	TROP	28-30	36.4	42.4-78				4
		Acanthurus chirurgus	TROP	28-30	35.9	40.5-				4
		Acanthurus coeruleus	TROP	28-30	35.3	35.1-				4
		Naso brevirostris	TROP	28-30	31.9	55.5-				4
		Naso brevirostris	TROP		Settlement	61.2			26.9	9
		Naso lituratus	TROP		Settlement				29.5	9
	Apogonidae	*Apogon sp b*	TROP		Settlement				5.1	9
		Apogon exostigma	TROP	28-30	21.4	16.2-43				4
		Apogon taeniophorus	TROP		Settlement				1.8	9
		Apogon trimaculatus	TROP	28-30	15.5	10-39.8			7.7	4, 9
		Apogon sp x	TROP	28-30	Settlement	25.6			11.8	9
		Apogon sp s	TROP	28-30	Settlement	20.7			6	9
		Apogonid sp A	TROP	28-30	12.1	12-22.7				4
		Apogonid sp B	TROP	28-30	9.7	8.5-27.4				4
		Apogonid sp C	TROP	28-30	11.6	11.8-				4
		Apogonid sp D	TROP	28-30	15.3	10-29.1				4

Order	Family	Species	Region	T (ºC)	Size range (mm)	Swimming measure				Reference
						Ucrit	Routine	Endurance	*In situ*	
		Apogonid sp E	TROP	28-30	12.7	5.9-22.5				4
		Apogonid sp F	TROP	28-30	14.2	9.1-34.3				4
		Apogonid sp G	TROP	28-30	9.5	8.5-21.5				4
		Fowlerias sp	TROP	28-30	11.1	10.9-				4
		Noemia octospina	TROP	28-30	14.6	22.4-				4
		Phaeoptyx pigmentaria	TROP	28-30	15.8	14.4-				4
		Sphaeramia nematoptera	TROP	27.5-29	Hatching-settlement	1.8-10.9	10-20			17, 26
	Balistidae	*Balistid sp.*	TROP	28-30	24.3	51.5				4
	Blenniidae	*Stanulus seyschellensis*	TROP		Settlement				26.4	9
		Exsenius stictus	TROP	28-30	19	0.3-11.1				4
		Caranx ignobilis	TROP	25	8-18	12-40		5-40	4-20	19
		Trachinotus blochii	TROP	25	4-6	7-16				7
		Trachurus sp.	TEMP	14.8-20.5	12.3–46.8			0–184.2		10
		Pseudocaranx dentex	TEMP		20.0			81.9		10
	Carapidae	*Carapini*	TROP	28-30	Settlement				20.4	9
	Chaetodontidae	*Chaetodon aureofasciatus*	TROP	28-30	16.8	43.8-			19	4, 9
		Chaetodon auriga	TROP	28-30	21.2	26.7-			7	4, 9
		Chaetodon citrinellus	TROP		Settlement				24.2	9
		Chaetodon melanotus	TROP	28-30	21.6	9.9				4
		Chaetodon plebeius	TROP	28-30	28-30	28.4-			25.5	4, 9
		Chaetodon rainfordi	TROP	28-30	14.4	34.7-			25.8	4, 9
		Chaetodon trifascialis	TROP	28-30	13.6	62.5			17.1	4, 9
		Chaetodontid sp	TROP	28-30	21.2	41.4				4
		Chelmon rostratus	TROP		Settlement				17.8	9
		Coradion chrysozonus	TROP		Settlement				26.1	9
	Ephippidae	*Platax teira*	TROP	25	4-11	5-20			3.7-20.1	7, 11
		Platax pinnatus	TROP		Settlement				4.9	9
		Myripristis sp	TROP		Settlement				65.5	9
		Neoniphon sammara	TROP		Settlement				48.1	9

Order	Family	Species	Region	T (ºC)	Size range (mm)	Swimming measure				Reference
						Ucrit	Routine	Endurance	*In situ*	
		Neoniphon argenteus	TROP		Settlement				7.2-62.3	8, 9
		Sargocentron coruscum	TROP	28-30	36.7	54.7-81				4
		Sargocentron vexillarium	TROP	28-30	37.6	80.2-				4
	Leiognathidae	*Leiognathus equulus*	TROP	25	5-16	1-31			6-25	7, 13
	Lethrinidae	*Lethrinus spp*	TROP	28-30	21.8	15.8-			19.4	4, 9
	Lutjanidae	*Lutjanus malabaricus*	TROP	25	5-29	1-50			6-30.2	7, 11
		Caesio cuning	TROP	28-30	21.9	34.2-			33.9	4, 9
		Lutjanus analis	TROP	28-30	30.7	37.9-				4
		Lutjanus apodus	TROP	28-30	21.3	40.4-				4
		Lutjanus argentimaculatus	TROP	25-30	5-22		3-20			29
		Lutjanus carponotatus	TROP	28-30	29.1	35.2-67			24.2	4, 9
		Lutjanus cyanopterus	TROP	28-30	32.3	45.3				4
		Lutjanus fulviflamma	TROP		Settlement	53.3			14.5	9
		Lutjanus quinquelineatus	TROP	28-30	26.1	27.6-				4
		Ocyurus chrysurus	TROP	28-30	23.4	42-42.6				4
		Ptereleotris heteroptera	TROP		Settlement				32.8	9
	Monocanthidae	*Oxymonacanthus longirostris*	TROP	28-30	23.3	29.3-31-			23.3	4, 9
		Paramonacanthussp.	TROP	28-30	23.1	4.6-79.4				4
		Parika scaber	TEMP	15-20	19.5–23.0			7.3–119.7		10
		Pseudomonacanthus peroni	TROP	28-30	27.6	18.1-				4
		Stephanolepis setifer	TROP	28-30	19.4	31.3				4
	Mullidae	*Upeneus tragula*	TROP	28-30	47	75.4				4
		Upeneichthys lineatus	TEMP	14.8-20.5	28.7–36.4			25.3–92.3		10
	Nemipteridae	*Scolopsis bilineatus*	TROP	28-30	16.2	26.9-60			12.9	4, 9
		Scolopsis sp	TROP	28-30	17.5	15.6-39-			10.5	4, 9
	Nomeidae	*Psenes sp.*	TROP	28-30	20	5.9-42.9				4
	Percichthyidae	*Macquaria novemaculeata*	TEMP	18-21	5-10.4	10-23				14
		Macquaria novemaculeata	TEMP	18-21	4.0-10.0			0-12.5		14
		Morone saxatilis	TEMP	22-25	4.2-7.2	0.5-2.64	6.1-8.9			15, 16

Order	Family	Species	Region	T (ºC)	Size range (mm)	Swimming measure				Reference
						Ucrit	Routine	Endurance	*In situ*	
	Polynemidae	*Eleutheronema tetradactylum*	TROP	25	7-22	3-35			3-18	7, 13
	Pomacanthidae	*Pomacanthus sexstriatus*	TROP	28-30	16.4	5.6-26.9			17.4	4, 9
	Pomacentridae	*Pomacentrid sp. A*	TROP	28-30	11.1	17.5–26.				4
		Pomacentrid sp. B	TROP	28-30	12.5	16.5–27.				4
		Abudefduf semptemfasciatus	TROP		Settlement				10.2	9
		Abudefduf vagiensis	TROP	28-30	17.4	26.7–73.			11.6	4, 9
		Acanthochromis polyacanthus	TROP	28-30	12.0	0.2–35.8				4
		Amblypomacentrus breviceps	TROP	28-30	12.6	21.2–44.			12.2	4, 9
		Amblyglyphidodon curacao	TROP		8.7-9.2	21-44.6		10.8-69	13.6	7
		Amphiprion clarkii	TROP	28-30	10.4	34.7				4
		Amphiprion melanopus	TROP	27.5-29	4.5-7.9	4.6-14.6	20-40			17, 26
		Chromis atripectoralis	TROP	28-30	10.3-10.8	21.8–61.		15.4	25.8	4, 9, 18
		Chromis viridis	TROP		Settlement				9.1-32.8	8, 9
		Chrysiptera glauca	TROP		Settlement				24.4	9
		Chrysiptera leucopoma	TROP		Settlement				34.6	9
		Chrysiptera rollandi	TROP	28-30	11-12.2	16.9–44.		18.1	11.7	4, 9
		Dascyllus aruanus	TROP	28-30	9.4	19.7–27.			24	4, 9
		Dascyllus reticulatus	TROP		Settlement	32.4			16.8	9
		Dascyllus trimaculatus	TROP	28-30	14.1	31.0–33.			13.7	4, 9
		Neopomacentrus azysron	TROP	28-30	17.2	31.9–39.			22	4, 9
		Neopomacentrus cyanomos	TROP	28-30	14-16.4	30.7–45.		31.4	22.6	4, 9, 18
		Pomacentrus amboinensis	TROP	28-30	12.1-14.9	22.0–68.		22.5	10.8	4, 9, 18
		Pomacentrus amboinensis	TROP	27.5-29	Hatching-settlement	3.5-30.3				17
		Pomacentrus amboinensis	TROP		2.6-13.3		10-70			26
		Pomacentrus brachialis	TROP	28-30	16.1	33.5–45.				4
		Pomacentrus chrysurus	TROP	28-30	16.4	19.5–54.				4
		Pomacentrus coelestis	TROP	28-30	19.8	35.5–62.			29.1	4, 9
		Pomacentrus lepidogenys	TROP	28-30	16.9	26.3–88.			28.1	4, 9
		Pomacentrus moluccensis	TROP	28-30	14.5	18.1–59.			12	4, 9

Order	Family	Species	Region	T (ºC)	Size range (mm)	Swimming measure				Reference
						Ucrit	Routine	Endurance	*In situ*	
		Pomacentrus nagasakiensis	TROP	28-30	13.3-16.9	25.5–83.		36		4, 18
		Pomacentrus wardi	TROP	28-30	16.6	42.6–62.				4
		Pristotis obtusirostris	TROP		28.3	41.8–88.			43.1	4, 9
		Stegastes sp	TROP		Settlement				25.9	9
		Stegastes diencaeus	TROP	28-30	15.6	33.0–47.				4
		Stegastes leucostictus	TROP	28-30	13.3	16.7–54.				4
		Stegastes partitus	TROP	28-30	17.2	34.5–63.				4
		Pseudochromid sp. B	TROP	28-30	17.8	21.3–42.				4
		Pseudochromid sp. C	TROP	28-30	17.1	8.2–31.5				4
	Sciaenidae	*Argyrosomus japonicus*	TEMP	22.6-24	3.5-14	4-13		0-12.5	2.5-8.4	14, 27
		Sciaenops ocellatus	TEMP	27.2	3.0-19.1	1.1-20.5				**Chapter III-B**
		Sciaenops ocellatus	TEMP	25-27	3-23.4		1.2-80.9			20
	Scombridae	*Scomber japonicus*	TEMP	19	4-15		4.6-56			31
	Serranidae	*Epinephelus coioides*	TROP	25	3-21	1-40			5.7-30.1	7, 11
		E. fuscoguttatus	TROP	25	12-27	10-50			1.7-27.2	7, 11
		E. malabaricus	TROP	25	3-25	1-64				7
		Diploprion bifasciatum	TROP		Settlement				21.8	9
		Plectropomus leopardus	TROP	28-30	21.4	13.2–43.			13	4, 9
	Siganidae	*Siganus spp.*	TROP	28-30	29.5	34.2–87.				4
	Sparidae	*Acanthopagrus australis*	TEMP	14.1-16.2	9.6–11.1				4–13	12
		Acanthopagrus australis	TEMP	21-23	4.9–10.7	2–26				14
		Acanthopagrus australis	TEMP		7.2–11.5				3–11.9	27
		Acanthopagrus schlegeli	TEMP		5.0–11.0				1–3	28
		Diplodus capensis	TEMP	17-20	8.9–16.0	2.8–35.2		0.2–32.4		22
		Pagrus auratus	TEMP		7.0–11.5				1–12.4	27
		Pagrus major	TEMP		5.0–7.5				1–4	28
		Rhabdosargus sarba	TEMP	14.1-16.2	9.3–11.				3.5–11	12
		Sarpa salpa	TEMP	17-20	12.1–21.3	4.8–33.4		0.07–64.8		22
		Sparus aurata	TEMP	15-28	16.1-28.4	9.1-17.5				23

Order	Family	Species	Region	T (ºC)	Size range (mm)	Swimming measure				Reference
						Ucrit	Routine	Endurance	*In situ*	
		Sparus aurata	TEMP	20.6-22.6	6.2-15.7	3.0 - 19.	0.9 - 17.2			**Chapter IV-A**
		Sparus aurata	TEMP	20.6-22.6	15.7			10.3-23.9		**Chapter IV-A**
	Sphyraenidae	*Sphyraena sp.*	TROP	28-30	23.0	21.5–38.				4
	Tetraodontidae	*Canthigaster bennnetti*	TROP	28-30	32.6	1.5–68.3			25.2	4, 9
		Canthigaster valentini	TROP		Settlement				14.4	9
	Terapontidae	*Terapon theraps*	TROP	28-30	19.2	25.8–72.				4
Pleuronectiformes	Pleuronectidae	*Pleuronectes platessa*	TEMP	7	5-13.5		6-16			24
	Pleuronectidae				6-10		4-10			1
	Paralichthiydae		TEMP				5-30			2
	Soleidae		TEMP				5-13			2
		Solea senegalensis	TEMP	21	3.5-7.5	0-5				**Chapter IV-B**
Scorpaeniformes	Cottidae	*Myoxocephalus scorpius*	TEMP	3-6	1.05-2.19	5.5-10.5				6
	Scorpidae	*Scorpis lineolatus*	TEMP	14.8-20.5	24.5–29.9			141.4–271.7		10
	Scorpaenidae	*Sebastes mystinus*	TEMP	12	4.61 (hatch)		3.14			25
		Sebastes carnatus	TEMP	12	5.25 (hatch)		3.25			25
		Sebastes atrovirens	TEMP	12	5.14 (hatch)		4.13			25