Resumo

O presente estudo descreve o efeito da inanição na sobrevivência de larvas recém eclodidas de *Lysmata amboinensis, L. debelius, L. boggessi* e *L. seticaudata*. As espécies estudadas ocupam diferentes habitats e exibem diferentes níveis de tolerância à ausência de alimento. Apenas as larvas recém eclodidas de *L. seticaudata* e *L. boggessi* apresentam lecitotrofia primária facultativa, realizando a muda do estádio de zoé I para zoé II em inanição. Todas as espécies estudadas apresentam lecitotrofia secundária facultativa no segundo estádio de zoé, sendo estas capazes de transitar para o estádio de zoé III na ausência de alimento. Contudo, esta capacidade apenas foi observada em larvas de zoé II que não tinham sido expostas à inanição em zoé I. A lecitotrofia secundária facultativa não tinha sido anteriormente registada para os camarões carídeos. As larvas recém eclodidas de *L. amboinensis, L. debelius, L. ankeri* e *L. seticaudata* toleram elevados períodos de inanição quando mantidas no escuro, comparativamente aos fotoperíodos de 12 e 24 h de luz. Este facto deve-se ao menor consumo energético das larvas, consequente do descréscimo na actividade natatória. A exposição à luz não é um factor determinante na captura das presas para as larvas de *Lysmata,* reforçando a assumpção de que as larvas de crustáceos não são predadores activos. As larvas recém eclodidas das diferentes espécies estudadas ingerem quantidades similares de nauplius de *Artemia*, enquanto *L. ankeri* e *L. seticaudata* são capazes de predar um maior número de metanauplius de *Artemia* enriquecidos. As características morfológicas e biométricas mostram-se insuficientes para explicar as diferenças observadas no número de presas ingeridas. A capacidade de captura e ingestão de presas de maiores dimensões por parte destas larvas (ex. *Artemia* metanauplius) abre boas perpectivas para a utiliação de dietas inertes durante os primeros estádios de zoé.

Palavras-chave: *Lysmata*, Resistência à inanição, Comportamento alimentar, Morfologia larvar, Camarões ornamentais.

Abstract

The present study describes the effect of starvation on the survival of early zoeal stages of *Lysmata amboinensis, L. debelius, L. boggessi, L. seticaudata*. Studied species occupy different habitats and exhibit different levels of tolerance to the absence of food. Only newly hatched larvae of *L. seticaudata* and *L. boggessi* display facultative primary lecithotrophy, moulting from zoea I to zoea II when starved. All studied species show secondary facultative lecithotrophy on the second zoeal stage, being able to moult to zoea III in the absence of food. Nevertheless, this ability was only recorded in larvae at the second zoeal stage which have not been previously exposed to starvation in zoea I. This is the firts record of secondary facultative lecithotrophy among caridena shrimps. Newly hatched larvae of *L. amboinensis, L. debelius, L. ankeri* e *L. seticaudata* show a higher tolerance to starvation periods when kept in the dark, compared to photoperiods with 12 or 24 h of light. This fact may be due to a lower larval energetic consumption as a consequence of a decreased swimming activity. Light exposure is not a determinant factor for prey capture in *Lysmata* larvae, reinforcing the assumption of decapod crustacean larvae not being active predators. Newly hatched larvae of different studied species ingest similar amounts of *Artemia* nauplii, while *L. ankeri* and *L. seticaudata* are able to capture a higher number of enriched *Artemia* metanuplii. The comparison of morphological and biometrical larval features do not show a particular pattern, being insufficient to explain the differences recorded in the number of ingested preys. The ability displayed by these organisms to capture and ingest large sized preys (e.g. *Artemia* metanauplii) opens good perspectives for the use of inert diets during early zoeal stages.

Key-words: *Lysmata*, Starvation Resistence, Feeding behavior, Larval morphology, Marine ornamental shrimps.

Introdução

Nas últimas décadas o desenvolvimento do comércio de organismos marinhos ornamentais cresceu significativamente, movimentando anualmente um valor estimado em mil milhões de Euros (Wabnitz *et al.,* 2003). Estima-se que 98% dos organismos transaccionados no mercado da aquariofília marinha sejam ainda capturados do seu ambiente natural, maioritariamente em recifes de coral (Thoney *et al.,* 2003). De forma a minimizar o impacto desta actividade, o desenvolvimento de protocolos de cultivo de espécies ornamentais sujeitas a maior exploração comercial tem aumentado. Esta estratégia é actualmente aceite como uma das formas mais eficazes para minimizar o problema da pressão exercida sobre estes ecossistemas pela indústria da aquariofília marinha (Arvelund *et al.,* 2003). Contudo, a oferta de organismos criados em cativeiro só será possível se forem desenvolvidas metodologias de cultivo que assegurem produções em grande escala e de forma rentável (Calado *et al.,* 2003a, b). Apesar dos peixes de recife de coral constituírem o grupo de organismos mais comercializado por parte desta indústria, os camarões ornamentais do género *Lysmata* têm adquirido na última década uma popularidade crescente (Calado *et al.,* 2003a). Além de serem muito atractivos, alguns destes camarões removem os ectoparasitas alojados nos peixes (Debelius, 2001; Spotte, 1998; Bauer, 2004), desempenhando um papel fundamental nas comunidades onde se inserem (Grutter, 1994) e afectando a diversidade de peixes existentes nos recifes (Becker & Grutter, 2004). Os camarões do género *Lysmata* destacam-se pela sua coloração, podendo atingir os 40 euros por indivíduo (Calado *et al.,* 2003a). Algumas espécies mais populares no mercado da aquariofilia marinha são: o camarão limpador listado do Indo-Pacífico *L. amboinensis* (De Man, 1888), o camarão fogo *L. debelius* Bruce, 1983; e os camarões "peppermint" do complexo *wurdemanni: L. boggessi* Rhyne & Lin, 2006 e *L. ankeri* Rhyne & Lin, 2006. O camarão do Mónaco *L. seticaudata* (Risso, 1816), com distribuição no Atlântico Nordeste e Mediterrâneo, apareceu recentemente no mercado Europeu como espécie alternativa às anteriormente descritas no complexo *wurdemanii*

(Calado *et al.,* 2001; Calado *et al*. 2003a). Esta espécie é particularmente importante na aquariofilia marinha Europeia pela sua capacidade de tolerância a conspecíficos, pelo comportamento limpador e no controlo da anémona vidro *Aiptasia pallida* Verrill, 1864 (Calado & Narciso*,* 2005; Delbeeck & Sprung, 2005). As anémonas vidro do género *Aiptasia* são organismos altamente resistentes com elevada capacidade regenerativa, constituindo verdadeiras "pragas" quando introduzidas acidentalmente nos aquários de recife, e provocando danos muitas vezes letais às espécies de corais através de processos alelopáticos (Trench, 1993).

O cultivo de camarões ornamentais apresenta numerosos constrangimentos a nível da maturação de reprodutores, do cultivo larvar e do crescimento de juvenis. A optimização de dietas de maturação (que assegurem uma boa qualidade/quantidade larvar), o fornecimento de presas vivas adequadas ao longo do desenvolvimento larvar (como microalgas, rotíferos, copépodes, nauplius de *Artemia* e metanauplius enriquecidos)*,* e a utilização de dietas de crescimento adequadas para juvenis, são pontos essenciais para ultrapassar as limitações impostas ao cultivo destas espécies (Calado *et al.,* 2003b; Palmtag & Holt, 2007).

Os camarões ornamentais do género *Lysmata* integram-se na seguinte classificação taxonómica (segundo Martin & Davis, 2001):

Reino: *Animalia*
Filo: *Arthropoda*
Subfilo*: Crustacea ,* Brünnich, 1772
Classe: *Malacostraca,* Latreille, 1802
Ordem: *Decapoda,* Grobben, 1892
Infra-ordem: *Caridea,* Dana, 1852
Familia: *Hippolytidae,* Dana, 1852
Género: *Lysmata,* Risso, 1816

O género *Lysmata* está presente em todos os mares tropicais do globo, nomeadamente nos recifes de coral do Indo-Pacífico e Caraíbas. No entanto, ocorre igualmente nas costas

rochosas das águas temperadas dos oceanos Atlântico e Pacífico (Debelius, 2001; Udekem d ´Acoz, 2000) (figura 1).

Figura 1. Indivíduos adultos do género *Lysmata*: a) *Lysmata seticaudata,* b) *L. boggessi/L. ankerii,* c) *L. debelius* d) *L. amboinensis.*

Os camarões do género *Lysmata* diferenciam-se de todos os outros crustáceos decápodes por apresentarem um sistema sexual único: hermafroditismo protândrico simultâneo (Bauer & Holt, 1998; Fiedler, 1998; Bauer, 2000; Zhang & Lin 2005; Baeza *et al.*, 2007ab). Este sistema caracteriza-se pelos indivíduos maturarem inicialmente como machos funcionais e mais tarde, com o crescimento (Bauer, 2006), transitarem para uma fase de hermafroditas simultâneos, (Bauer, 2000), não podendo contudo, realizar auto-fecundação (Bauer & Holt, 1998; Fiedler, 1998; Bauer & Newman, 2004). Na fase de hermafroditas simultâneos, os camarões podem copular durante qualquer período como machos, mas apenas podem acasalar como fêmeas após a realização da *ecdisys* ou muda (Lin & Zhang, 2001). Este sistema sexual foi confirmado inicialmente apenas para *L. wurdemanni* (Bauer & Holt, 1998)

e *L. amboinensis* (Fiedler, 1998), baseando-se na descrição da sua morfologia reprodutiva. No entanto, Bauer (2000) advoga que este sistema sexual pode abranger todos os indivíduos do género *Lysmata.* A mediação social (densidade) foi documentada como o factor mais importante na alteração do sexo em *L. wurdemanni* (Lin & Zhang, 2001; Baeza & Bauer, 2004) e *L. seticaudata* (Calado *et al.*, 2007), no entanto, outros factores abióticos como a temperatura, o fotoperíodo (Bauer, 2002) e a dieta (Zupo, 2000) podem afectar a mudança de sexo. O tamanho do indivíduo pode também ser condicionante na alteração de sexo (Noël, 1973).

Nas condições óptimas de temperatura e fotoperíodo, os indivíduos que se encontram na fase de hermafroditas simultâneos incubam os ovos no abdómen, durante 12 a 15 dias. Após a eclosão das larvas, o progenitor faz a muda e pode acasalar novamente desempenhando o papel de "fêmea" (Lin & Zhang, 2001).

O desenvolvimento larvar do género *Lysmata* é caracterizado por uma sequência de estádios distintos (entre 9 a 13 estádios) (Calado *et al*., 2004). Estas fases são intercaladas pela muda (Anger, 1987), e estão sujeitas a alterações morfológicas, fisiológicas e bioquímicas profundas (Chang, 1995). Deste modo, durante o seu ciclo de vida, as larvas destes organismos passam por diversos estádios com comportamentos alimentares característicos (Phlippen *et al.,* 2001). Quaisquer alterações na quantidade/qualidade de alimento ingerido podem induzir efeitos deletérios. A capacidade destes organismos sobreviverem e recuperarem de longos períodos de ausência de alimento é fundamental (Brzek & Konarzewski, 2001), reflectindo a resistência à inanição e a capacidade dos organismos armazenarem e controlarem os gastos energéticos quando sujeitos a limitações extremas (Wang *et al.,* 2006).

As larvas dos crustáceos decápodes dispõem de requisitos nutricionais que variam intra e inter-especificamente, assim como sazonal e anualmente (Anger, 2001). Nos primeiros estádios de desenvolvimento, estes requisitos estão dependentes dos aspectos qualitativos e

quantitativos das reservas armazenadas no embrião (Kattner *et al.,*1994). Anger (2001) verificou que na maioria das espécies de decápodes as reservas vitelinas existentes nos embriões, provenientes do investimento parental, são catabolizadas quase na sua totalidade durante o desenvolvimento embrionário. Assim, o fornecimento de alimento adequado após a eclosão desempenha um papel crucial para a sobrevivência larvar (Simões *et al.,* 2002).

Anger & Dawirs (1981) demonstraram a existência de um período crítico no desenvolvimento larvar dos crustáceos decápodes designado por "ponto de não retorno" (PNR). Este conceito traduz um limite para o período de ausência de alimento a que a larva é sujeita, para além do qual esta perde a capacidade de recuperar do *stress* nutricional imposto, culminando invariavelmente na sua morte.

Segundo Anger (2001), quando as larvas de crustáceos são submetidas a períodos de inanição podem ser identificadas três fases de degradação da biomassa. Inicialmente, as reservas lipídicas são mobilizadas, reflectindo um decréscimo na razão entre os lípidos e as proteínas, típico de privações relativamente curtas de alimento. Quando grande parte das reservas lipídicas é consumida, à excepção daquelas que desempenham funções na estrutura das membranas celulares, as proteínas começam a ser utilizadas. Esta fase, maioritariamente caracterizada pelo catabolismo proteico, contribui para o aumento da razão existente entre lipídos e proteínas (resultante da degradação de músculo e tecido nervoso). Numa fase final do período de inanição, os lípidos estruturais podem igualmente ser degradados promovendo um novo decréscimo da razão entre lipídos e proteínas. Nestas condições, a larva atinge o PNR, não recuperando do *stress* nutricional, mesmo na presença de alimento adequado (Abrunhosa & Kittaka, 1997; Ritar *et al.,* 2003). A compreensão do PNR é uma ferramenta importante para a optimização dos protocolos de cultivo das espécies de crustáceos decápodes comercialmente importantes (Paschke *et al.,* 2004). Este parâmetro pode igualmente ser utilizado como índice comparativo da vulnerabilidade nutricional das diferentes espécies e/ou estádios larvares (Gimenez & Anger, 2005).

A inanição afecta negativamente as larvas de *Lysmata* (ex. indução de mortalidade; adiamento da metamorfose) (Calado & Narciso, 2005; Calado *et al.,* 2005), pelo que deverá ser evitada ou minimizada sempre que possível (Simões *et al.,* 2002). A privação de alimento a larvas recém-eclodidas de *Lysmata* tem sido justificada, erradamente, através da assumpção de que todas as larvas de decápodes apresentam lecitotrofia primária facultativa. As larvas com esta capacidade possuem reservas vitelinas suficientes para transitar para o segundo estádio de zoé em condições de *stress* nutricional (Thessalou-Legaki *et al.,* 1999), inclusive a ausência total de alimento. Contudo, nem todas as espécies de decápodes exibem esta capacidade (Calado *et al.*, 2007). As respostas dos crustáceos face à inanição parecem ser determinadas pelo tipo de desenvolvimento larvar, podendo ocorrer uma alteração do substrato energético catabolizado, de acordo com a espécie e estádio de desenvolvimento larvar (Sanchéz-Paz *et al.,* 2006). Apesar da informação disponível referente aos requisitos lipídicos e proteicos de larvas de crustáceos sujeitas a inanição ser contrastante, os lípidos são geralmente considerados como a maior fonte energética nas larvas de camarões carídeos. Estes compostos estão envolvidos em processos essenciais para o crescimento, sobrevivência nas primeiras fases larvares, processos de muda e reprodução (Anger *et al*., 1998).

Os efeitos da alimentação e da inanição no desenvolvimento das larvas de *Lysmata* não foram ainda estudados em detalhe, nomeadamente a acumulação e perda de nutrientes essenciais. No entanto, podem revelar-se como indicadores dos recursos energéticos utilizados para cada estádio de desenvolvimento (Sanchez-Paz *et al.,* 2006) e têm aplicabilidade na optimização dos processos de cultivo.

A fim de prevenir períodos de ausência de alimento, é importante colocar ao dispor de larvas recém eclodidas, presas alimentares adequadas. O conhecimento do desenvolvimento larvar de espécies próximas é muito importante do ponto de vista da ecologia alimentare das suas relações filogenéticas (Porter *et al*., 2005).

Para além de compreender como se comportam as larvas de *Lysmata* face à inanição,

torna-se também importante conhecer a morfologia dos apêndices relacionados com a captura e manipulação do alimento. Este aspecto pode ser um factor preponderante para o sucesso do cultivo das larvas de crustáceos decápodes, uma vez que pode sugerir qual o tipo de presas mais indicado para cada estádio de desenvolvimento (Crain, 1999; Cox & Johnston, 2003; Epelbaum & Borisov, 2006). As larvas de crustáceos decápodes cultivadas comercialmente exibem uma grande variedade de estratégias alimentares. A maioria dos crustáceos decápodes faz a incubação dos ovos no abdómen e liberta uma zoé em estado avançado de desenvolvimento, que revela ser totalmente ou parcialmente planctotrófica na primeira alimentação (Jones *et al*., 1997). As larvas capturam as partículas alimentares com o auxílio das maxílulas, maxilas e maxilípedes, sendo estas posteriormente mastigadas pelas mandíbulas e conduzidas até às estruturas internas do tracto digestivo. De facto, estas estruturas não diferem significativamente quando se comparam os primeiros estádios larvares e os indivíduos adultos, embora possam ocorrer alterações ontogénicas na sua funcionalidade (Anger, 2001).

Os trabalhos actualmente existentes para as fases larvares do género *Lysmata* descrevem os caracteres morfológicos (Calado *et al*., 2004) e analisam a cinemática alimentar (Rhyne *et al.*, 2001), não havendo até ao momento quaisquer estudos integrando dados de morfologia e comportamento alimentar em larvas de *Lysmata.*

As respostas comportamentais das larvas de crustáceos decápodes à luz são muito complexas e variáveis (Forward & Buswell, 1989) resultando da combinação da fototaxia e dos comportamentos natatórios exibidos pelas larvas recém eclodidas. O fotoperíodo é um parâmetro facilmente manipulável em laboratório e influencia a sobrevivência e o crescimento no cultivo de larvas de crustáceos (Bermudes & Ritar, 2008). Nas larvas dos crustáceos decápodes a luz pode induzir ao estímulo da actividade natatória (Sulkin, 1984). Os efeitos da luz no comportamento alimentar das larvas de *Lysmata* são ainda desconhecidos, contudo, para outras espécies de crustáceos, como *Jasus edwardsii* (Hutton, 1875), observam-se

maiores taxas de crescimento a baixa intensidade luminosa (Moss *et al.,* 1999).

O presente trabalho teve como objectivo avaliar a capacidade predatória dos primeiros estádios de zoés de camarões ornamentais do género *Lysmata,* mais concretamente de *Lysmata seticaudata, L. boggessi*, *L. debelius, L. amboinensis* e *L. ankeri* sendo estas espécies de regiões biogeográficas distintas e ocupando diferentes tipos de habitat.

Simultaneamente, estimou-se o efeito de diferentes regimes de fotoperíodo nas taxas de ingestão larvar e comparou-se a morfologia dos apêndices envolvidos na captura e manipulação de alimento. Por fim, determinou-se o nível de tolerância das diferentes larvas de *Lysmata* quando submetidas a períodos de ausência de alimento.

Seguidamente, são apresentados os diferentes aspectos abordados neste trabalho, dividido em dois capítulos: I. Resistência à inanição de larvas de camarões ornamentais do género *Lysmata*, II. Importância da luz e morfologia larvar na resistência à inanição e capacidade de alimentação de larvas recém eclodidas de camarões ornamentais do género *Lysmata.*

CAPÍTULO I

1. Resistência à Inanição de larvas de camarões ornamentais do género *Lysmata*

Este capítulo foi publicado sob formato de artigo científico na revista internacional "Journal of Experimental Marine Biology and Ecology, Vol. 351, Issues 1-2, pp. 226-233", no ano de 2007, com o título:

"Starvation resistance of early zoeal stages of marine ornamental shrimps from different habitats of the genus *Lysmata* (Decapoda: Hippolytidae)."

tendo como co-autores : Ricardo Calado, Gisela Dionísio, Maria Teresa Dinis.

Starvation resistance of early zoeal stages of marine ornamental shrimps *Lysmata* spp. (Decapoda: Hippolytidae) from different habitats

Ricardo Calado, Gisela Dionísio and Maria Teresa Dinis

Abstract

The genus *Lysmata* is present worldwide in a variety of habitats and newly hatched larvae from different species may display variable tolerance to starvation. This work evaluates the effect of starvation on survival of newly hatched and zoea II larvae of *Lysmata amboinensis*, *L. boggessi*, *L. debelius* and *L. seticaudata*. Survival of newly hatched larvae was influenced by the period of starvation and by parental species. *Lysmata boggessi* and *L. seticaudata* newly hatched larvae displayed facultative primary lecithotrophy (FPL), with all continuously starved larvae molting to zoea II. In general, when starved for similar periods, *L. debelius* larvae displayed significantly higher survival to zoea II than *L. amboinensis*. However, no *L. debelius* or *L. amboinensis* larvae endured starvation for more than 24 hours. Survival from zoea II to zoea III was affected by the duration of starvation period, by parental species and by starvation of the first zoeal stage (for *L. boggessi* and *L. seticaudata*). Zoea II from all tested species, produced from starved or fed zoea I, displayed similar survival when larval preys were always available. However, when starved for only 24 hours, zoea II of *L. boggessi* and *L. seticaudata* originating from continuously starved zoea I displayed significantly lower survival than those produced from fed larvae. Only zoea II originating from fed zoea I displayed facultative secondary lecithotrophy (FSL) and larvae from all tested species were able to successfully molt to the third zoeal stage in the total absence of food. This feature has never been previously recorded among caridean shrimps. Larval preys ingested in zoea I contribute to the build up of energetic reserves that latter are catabolized during starvation in zoea II, allowing some larvae to molt to zoea III. Since differences were recorded among the survival displayed by starved zoea II of *Lysmata* with FPL (*L. boggessi* and *L. seticaudata*)

and those without such feature (*L. amboinensis* and *L. debelius*), it seems that egg yolk reserves are totally depleted in starved larvae and that energy accumulated through larval preys ingestion plays the crucial role. Future studies may help to confirm the current informal division of genus *Lysmata*: "crowd" species (*L. boggessi* and *L. seticaudata*) displaying FPL *vs* "pairs" species (*L. amboinensis* and *L. debelius*) without FPL. Additionally, it may also be confirmed that "pairs" species are ancestral to "crowd" species, since it is unlikely that FPL would have regressed to full planktotrophy.

Key words: *Lysmata*; marine ornamental shrimps; PNR; point of no return; starvation resistance; zoea

1. Introduction

Newly hatched decapod crustacean larvae are known to display dietary requirements varying intra and interspecifically, seasonally and annually (Anger, 2001). Such dietary requirements are dependent on the qualitative and quantitative aspects of energetic reserves stored in the egg yolk (Kattner et al., 1994). In general, since yolk reserves are rapidly catabolized, decapod larvae must start feeding immediately after hatching and avoid the deleterious effects induced by starvation. Anger and Dawirs (1981) demonstrated the existence of a critical period in the larval development of crustacean decapods termed as "point of no return" (PNR). Briefly, PNR represents a threshold where larvae which were exposed to starvation and were subsequently fed may remain alive for a variable period of time, although being incapable to recover from the nutritional stressed early imposed, not developing further and finally dying. The understanding of these critical points on early larval feeding can be a valuable tool for the establishment of successful aquaculture protocols for commercially valuable decapods (Paschke et al., 2004), helping to minimize early zoeal stages mortality and increasing survival to metamorphosis. The importance of such studies is even more relevant if we take into account that PNR can be quantified experimentally, helping to clarify the nutritional flexibility of planktothrophic decapod larvae (Sulkin and van Heukelem, 1980;

Sulkin et al., 1998; Gimenez and Anger, 2005).

In the latest years, the culture of marine ornamental shrimps of the genus *Lysmata* has been frequently addressed, in an attempt to establish commercial scale culture protocols that minimize the growing fishing efforts for these highly valuable organisms (Calado et al, 2003a, b). Early and late zoeal stages feeding have been pointed as the main bottlenecks impairing the successful culture of these ornamental shrimps (Rhyne and Lin, 2004). However, growing experimental evidence seem to indicate that late zoeal stage mortality and metamorphosis delay are heavily influenced by nutritional stress induced to early larval stages of ornamental shrimps (Simões et al., 2002; Calado et al., 2005a, b). Marine ornamental shrimp's larvae commonly hatch during the night and are left in the absence of food until the next morning. Therefore, newly hatched larvae are commonly exposed to starvation periods that may range at least from 6 to 12 hours. In extreme cases, due to the wrong believe that all marine shrimps hatch with enough yolk reserves that allow them to molt from zoea I to zoea II in the absence of food (facultative primary lecithotrophy (FPL), see Anger, 1995, 2001; Thessalou-Legaki et al., 1999), newly hatched larvae can be starved for up to 48 hours.

Since shrimps from the genus *Lysmata* occur worldwide in habitats ranging from coral reefs in tropical areas to rocky shores in warm temperate seas (Debelius, 2001), it is possible that newly hatched larvae may display different tolerance levels to variable starvation periods. The present work evaluates the effect of different periods of starvation on survival and stage duration of newly hatched and zoea II larvae of marine ornamental shrimps of the genus *Lysmata* occurring in different habitats: *L ysmata amboinensis* (De Man, 1888), from the shallower areas of coral reefs through the Indo-Pacific (Debelius, 2001); *L. boggessi* Rhyne and Lin, 2006, mainly living under rocky ledges at depths up to 15m in the Gulf of Mexico (Rhyne and Lin, 2006); *L. debelius* Bruce, 1983, commonly occurring at depths greater than 10 m in the coral reefs of the Indo-pacific (Debelius, 2001); and *L. seticaudata* (Risso, 1816), inhabiting rocky bottoms of the warm-temperate waters of the north-eastern Atlantic and the

Mediterranean sea, being present from the lower intertidal area to depths up to 60 m (Udekem d'Acoz, 1999).

2. Materials and methods

2.1. Parental broodstock keeping

Ten specimens of *Lysmata amboinensis*, *L. boggessi*, , *L. debelius* and *L. seticaudata* in simultaneous hermaphrodite sexual phase and displaying average total lengths (TL - measured from the anterior tip of the rostrum to the posterior end of the telson) of 50 ± 2 mm, were used to form five randomly assembled breeding pairs for each species. Each breeding pair was kept in a rectangular glass tank (0.60 m long x 0.30 m wide x 0.30 m high, total volume 54 L) connected to a recirculated maturation system for ornamental decapods described in detail by Calado et al. (2007). A photoperiod of 12h light: 12h dark was used, with breeding tanks being illuminated from above with fluorescent white lights. Artificial seawater was prepared using freshwater purified by a reverse osmosis unit and mixed with the salt Crystal Sea® produced by Marine Enterprises International® (Baltimore, MD, USA), following the instructions of the manufacturer. Salinity was daily checked and maintained at 35 ± 1, while temperature was kept at 25 ± 1ºC through the use of a heating/cooling unit. Ammonia and nitrite were maintained bellow detectable levels and nitrate and pH showed average values (± standard deviation, S.D.) of 3.1 (± 2.2) mg l^{-1} and 8.0 (± 0.1), respectively.

All breeding pairs were fed Marine Cuisine®, a commercial frozen diet for marine aquarium organisms produced by San Francisco Bay Brand® (Newark, CA, USA) and according to the manufacturer composed by *Artemia franciscana*, krill, mysid shrimp, menhaden oil, astaxanthin, sodium alginate, *Spirulina* and a vitamin premix (wheat flour, Vitamin A acetate, cholecalciferol (source of vitamin D3), vitamin B12 supplement, riboflavin, niacin, calcium pantothenate, folic acid, menadione sodium bisulfate complex, pyridoxine hydrochloride, thiamine mononitrate, biotin, inositol, L-ascorbyl-2-polyphosphate, betaine, d-alpha mixed

tocopherols (source of vitamin E)). The diet was fed on a dry-weight basis, with each breeding pair being fed 3 times per day (07.00, 14.00 and 21.00 h) in a combined total of 15% of each shrimp pair biomass.

In order to guarantee that only nutrients provided by the diet being used in the laboratory were mobilized for gonad maturation, assuring a common nutritional pool to larvae produced by all *Lysmsta* species, the first three embryo batches produced after pairing were discarded and not considered for the present study.

Due to the large number of larvae required to perform the present study, it was impossible to run all experimental trials simultaneously. In this way, newly hatched larvae were divided by as many replicates as possible from different treatments. The large number of shrimps producing larvae allowed us to overlap most replicates. Nonetheless, there were also some replicates from the same experimental trial that were separated by a maximum of 3 days.

2.2 Starvation resistance of newly hatched *Lysmata* spp. larvae

Newly hatched larvae from at least three parental shrimps, from each *Lysmata* species studied, were collected immediately after hatching and divided in groups of 60 individuals, with each larva being placed in a small individual plastic container (20ml each). The resistance to starvation experiments comprised the following groups of initial starvation followed by continuous feeding: larvae starved for 6 h, 12 h, 24 h, 48 h and 72 h after hatching. Additionally, 2 other groups of newly hatched larvae were continuously fed or starved after hatching (for 120 hours), being used as positive and negative control, respectively. Four replicates were used for each treatment, for a total of 7 treatments x 60 larvae x 4 replicates x 4 *Lysmata* species = 6720 larvae. Daily, the water from each larval container was 100% renewed by 1 μm filtered artificial seawater, with survival and ability to molt to the second zoeal stage also being checked for 5 days (at this time, all larvae were either at the next zoeal stage or dead). Temperature was kept at 25 ± 1ºC through a water bath connected to a heating/cooling system. Larvae were fed on newly hatched *Artemia* nauplii, supplied to the

larvae at a density of 2 larval preys ml^{-1}.

2.3 Starvation resistance of second zoeal stage *Lysmata* spp. larvae

Newly hatched larvae from at least three parental shrimps, from each *Lysmata* species studied, were placed in 20 l cylindrico-spherical larviculture tanks (adapted from those described by Calado et al. (2003c)). Two larviculture tanks were used for each *Lysmata* species, one being daily provided with newly hatched *Artemia* nauplii at a density of 2 larval preys ml^{-1} and the other not being provided any type of larval food. A preliminary study revealed that newly hatched *Lysmata* species do not cannibalize each other, assuring that larvae that were not being provided larval preys were truly in continued starvation. Larviculture tanks were checked daily for larvae reaching the second zoeal stage. Once larvae in zoea II were detected, they were removed from the larviculture tank and were pooled in groups of 60 continuously fed (or starved) larvae from each *Lysmata* species. Each larva was placed in an individual container (as described in the previous section) and the following treatments of initial starvation, followed by continuous feeding, were performed: larvae starved for 24 h, 48 h and 72 h after molting to zoea II. Again, 2 additional groups of newly molted zoea II larvae were continuously fed or starved, acting as positive and negative controls, respectively. Four replicates were used for each treatment, for a total of 5 treatments x 2 types of zoea II (produced from fed or starved zoea I) x 60 larvae x 4 replicates x 4 *Lysmata* species = 9600 larvae. Water from each larval container was also 100% daily renewed by 1 μm filtered artificial seawater, with larval survival and ability to molt to the third zoeal stage also being daily checked for 5 days (at this time, all larvae were either at the next zoeal stage or dead). Larval feeding and temperature control were performed as described in the previous section.

2.4. Statistical analysis

Larval survival and stage duration of newly hatched *Lysmata* spp. larvae starved during different periods of time after hatching was compared using a two-way analysis of variance. Survival, as well as larval stage duration, of the second zoeal stage of *Lysmata* spp. larvae,

previously fed or starved in zoea I, and exposed to different periods of starvation was compared using a multiway factorial analysis of variance MANOVA. Statistical analysis was performed using the software Statistica (version 6.0), with assumptions being verified prior to analysis and data transformations being performed when required (e.g. when analyzing percentages). Whenever significance was accepted, at $P < 0.05$, the Tukey multiple comparison test was used (Zar 1999).

3. Results

The analysis of variance revealed that different periods of starvation, as well as parental species, significantly affected the survival displayed by newly hatched larvae ($df = 6$, $F = 1348.16$; $P < 0.0001$ and $df = 3$, $F = 3852.91$; $P < 0.0001$, respectively). A significant interaction between starvation periods* parental species of *Lysmata* was also recorded ($df = 18$, $F = 459.8$; $P < 0.0001$). Survival of *L. boggessi* and *L. seticaudata* larvae to zoea II was not affected by the experimental starvation periods, with newly hatched larvae always being able to molt to the second zoeal stage with 100% survival (Table 1). The average survival (± standard deviation) to zoea II displayed by *L. amboinensis* and *L. debelius* larvae starved for 6 hours after hatching (95.0 ± 3.1% and 96.7 ± 3.2%, respectively) was not significantly different ($P > 0.05$) from that of *L. boggessi* and *L. seticaudata* (100% for both species). However, when starved for longer periods, *L. amboinensis* and *L. debelius* always displayed significantly lower survival than *L. boggessi* and *L. seticaudata* (Table 1). *Lysmata debelius* displayed significantly higher survival to the second zoeal stage than *L. amboinensis* when starved for 12 hours (86.7± 5.4% and 76.7± 3.8%, respectively) and 24 hours (76.7± 3.8% and 50.0 ± 8.6%, respectively). Neither *L. debelius* nor *L. amboinensis* newly hatched larvae could endure starvation periods longer than 24 hours (Table 1).

Table 1 – Average survival (%) (± standard deviation) of *Lysmata amboinensis*, *L. boggessi*, *L. debelius* and *L. seticaudata* newly hatched larvae to the second zoeal stage under different initial starvation periods (6, 12, 24, 48 and 72 hours of starvation after hatching - S_6, S_{12}, S_{24}, S_{48} and S_{72}) and continuously fed and starved larvae (FC and SC, respectively) (n = 4 replicates of 60 larvae per each treatment, per each species). Different superscript letters in the same row and different superscript numbers in the same column represent significant differences ($P < 0.05$). * Larvae had already molted to the next zoeal stage.

	L. amboinensis	*L. boggessi*	*L. debelius*	*L. seticaudata*
FC	$98.3 \pm 1.3^{a,1}$	$100.0 \pm 0.0^{a,1}$	$98.3 \pm 1.1^{a,1}$	$100.0 \pm 0.0^{a,1}$
S_6	$95.0 \pm 3.1^{a,1}$	$100.0 \pm 0.0^{a,1}$	$96.7 \pm 3.2^{a,1}$	$100.0 \pm 0.0^{a,1}$
S_{12}	$76.7 \pm 3.8^{a,2}$	$100.0 \pm 0.0^{b,1}$	$86.7 \pm 5.4^{c,2}$	$100.0 \pm 0.0^{b,1}$
S_{24}	$50.0 \pm 8.6^{a,3}$	$100.0 \pm 0.0^{b,1}$	$76.7 \pm 3.8^{c,3}$	$100.0 \pm 0.0^{b,1}$
S_{48}	$0.0 \pm 0.0^{a,4}$	$100.0 \pm 0.0^{b,1}$	$0.0 \pm 0.0^{a,4}$	$100.0 \pm 0.0^{b,1}$
S_{72}	$0.0 \pm 0.0^{a,4}$	*	$0.0 \pm 0.0^{a,4}$	*
SC	$0.0 \pm 0.0^{a,4}$	*	$0.0 \pm 0.0^{a,4}$	*

Zoea I duration was significantly affected by different starvation periods ($df = 6$, $F = 1158.29$; $P < 0.0001$) and parental *Lysmata* species ($df = 3$, $F = 72.55$; $P < 0.0001$). A significant interaction between starvation periods and parental species was also recorded ($df = 18$, $F = 387.53$; $P < 0.0001$). The first larval stage of *L. boggessi* and *L. seticaudata* displayed an average duration of 2 days, while *L. debelius* and *L. amboinensis* larvae always displayed a significantly ($P < 0.05$) longer duration (Table 2). *Lysmata debelius* and *L. amboinensis* larvae that were continuously fed or starved for 6 hours after hatching displayed significantly shorter ($P < 0.0001$) larval duration periods (2.7- 2.8 days), when compared to larvae starved for 12 or 24 hours after hatching (3.1-3.5 days). However, *L. boggessi* and *L. seticaudata* larvae, either fed continuously or starved for an equal period after hatching, always displayed similar duration periods ($P = 0.27$) (Table 2).

Table 2 – Average zoea I duration (days) (± standard deviation) of *Lysmata amboinensis*, *L. boggessi*, *L. debelius* and *L. seticaudata* under different initial starvation periods (6, 12, 24, 48 and 72 hours of starvation after hatching - S_6, S_{12}, S_{24}, S_{48} and S_{72}) and continuously fed and starved larvae (FC and SC, respectively) (n = 4 replicates of 60 larvae per each treatment, per each species). Different superscript letters in the same row and different superscript numbers in the same column represent significant differences ($P < 0.05$). * Larvae died before molting to the next zoeal stage. ** Larvae had already molted to the next zoeal stage.

	L. amboinensis	*L. boggessi*	*L. debelius*	*L. seticaudata*
FC	$2.7 \pm 0.0^{a,1}$	$2.0 \pm 0.0^{b,1}$	$2.7 \pm 0.1^{a,1}$	$2.0 \pm 0.0^{b,1}$
S_6	$2.8 \pm 0.2^{a,1}$	$2.0 \pm 0.0^{b,1}$	$2.8 \pm 0.2^{a,1}$	$2.0 \pm 0.0^{b,1}$
S_{12}	$3.4 \pm 0.2^{a,2}$	$2.0 \pm 0.0^{b,1}$	$3.1 \pm 0.2^{a,2}$	$2.0 \pm 0.0^{b,1}$
S_{24}	$3.5 \pm 0.3^{a,2}$	$2.0 \pm 0.0^{b,1}$	$3.3 \pm 0.1^{a,2}$	$2.0 \pm 0.0^{b,1}$
S_{48}	*	$2.0 \pm 0.0^{b,1}$	*	$2.0 \pm 0.0^{b,1}$
S_{72}	*	**	*	**
SC	*	**	*	**

Concerning the second zoeal stage, survival to zoea III was significantly affected by the different starvation periods ($df = 4$, $F = 2584.94$; $P < 0.0001$), by parental species ($df = 3$, $F = 1196.19$; $P < 0.0001$) and by the continued starvation or feeding of the first zoeal stage ($df = 1$, $F = 6557.38$; $P < 0.0001$). Significant interactions were detected between different starvation periods * parental *Lysmata* species ($df = 12$, $F = 115.23$; $P < 0.0001$), different starvation periods * continued starvation or feeding of the first zoeal stage ($df = 4$, $F = 304.44$; $P < 0.0001$), parental species * continued starvation or feeding of the first zoeal stage ($df = 3$, $F = 123.83$; $P < 0.0001$) and different starvation periods * parental species * continued starvation or feeding of the first zoeal stage ($df = 12$, $F = 239.96$; $P < 0.0001$). Zoea II from all tested species, produced from continuously starved or fed zoea I, displayed similar survival results when larval preys were always available (Table 3). However, even when only starved for 24 hours, zoea II of *L. boggessi* and *L. seticaudata* originating from starved zoea I displayed significantly lower survival (100 ± 0.0 % vs 66.7 ± 5.4%, for zoea II of *L. boggessi* originating from fed or starved zoea I, respectively; $P = 0.0001$) (100.0 ± 0.0% vs 65.0 ± 6.4%, for zoea II of *L. seticaudata* originating from fed or starved zoea I, respectively; $P = 0.0001$). A similar result was recorded for larvae starved for 48 hours, with zoea II of *L.*

boggessi and *L. seticaudata* originating from starved zoea I being unable to endure 72 hours or continued starvation (Table 3). Zoea II originating from fed zoea I, from all tested species, were able to successfully molt to the third zoeal stage in the total absence of food, displaying similar survival rates (10.0 ± 3.5%, 15.0 ± 6.4%, 15.0 ± 3.3%, 13.3 ± 5.2%, for *L. amboinensis*, *L. boggessi*, *L. debelius* and *L. seticaudata*, respectively; P = 1.00) (Table 3).

Table 3 – Average survival (%) (± standard deviation) of zoea II of *Lysmata amboinensis*, *L. boggessi*, *L. debelius* and *L. seticaudata*, produced from fed or starved zoea I, to the third zoeal stage under different initial starvation periods (24, 48 and 72 hours of starvation after hatching - S_{24}, S_{48} and S_{72}) and continuously fed and starved larvae (FC and SC, respectively) (n = 4 replicates of 60 larvae per each treatment, per each species) (zoea I fed – F; zoea I starved – S). Different superscript letters in the same row and different superscript numbers in the same column represent significant differences (P < 0.05). * Newly hatched larvae of this species could not molt to zoea II when continuously starved.

	L. amboinensis		*L. boggessi*		*L. debelius*		*L. seticaudata*	
	ZI F	ZI S	ZI F	ZI S	ZI F	ZI S	ZI F	ZI S
FC	$98.3 \pm 1.3^{a,1}$	*	$100.0 \pm 0.0^{a,1}$	$100.0 \pm 0.0^{a,1}$	$100.0 \pm 0.0^{a,1}$	*	$100.0 \pm 0.0^{a,1}$	$100.0 \pm 0.0^{a,1}$
S_{24}	$53.3 \pm 5.4^{a,2}$	*	$100.0 \pm 0.0^{b,1}$	$66.7 \pm 5.4^{c,2}$	$90.0 \pm 3.0^{d,2}$	*	$100.0 \pm 0.0^{b,1}$	$65.0 \pm 6.4^{a,2}$
S_{48}	$13.3 \pm 0.0^{a,3}$	*	$75.0 \pm 6.4^{b,2}$	$10.0 \pm 3.8^{a,3}$	$78.3 \pm 6.4^{b,3}$	*	$73.3 \pm 5.4^{b,2}$	$8.3 \pm 3.3^{a,3}$
S_{72}	$11.7 \pm 4.1^{a,3}$	*	$65.0 \pm 3.2^{b,3}$	$0.0 \pm 0.0^{c,4}$	$23.3 \pm 3.8^{d,4}$	*	$63.3 \pm 3.0^{b,3}$	$0.0 \pm 0.0^{c,4}$
SC	$10.0 \pm 3.5^{a,3}$	*	$15.0 \pm 6.4^{a,4}$	$0.0 \pm 0.0^{b,4}$	$15.0 \pm 3.3^{a,5}$	*	$13.3 \pm 5.2^{a,4}$	$0.0 \pm 0.0^{b,4}$

The average duration of the second zoeal stage was significantly influenced by starvation periods (df = 4, F = 378.51; P < 0.0001), parental species (df = 3, F = 564.61; P < 0.0001) and by continued starvation or feeding of the first zoeal stage (df = 1, F = 15382.22; P < 0.0001). Significant interactions were detected between different starvation periods * parental *Lysmata* species (df = 12, F = 199.35; P < 0.0001), different starvation periods * continued starvation or feeding of the first zoeal stage (df = 4, F = 1471.13; P < 0.0001), parental species * continued starvation or feeding of the first zoeal stage (df = 3, F = 1759.71; P < 0.0001) and different starvation periods * parental species * continued starvation or feeding of the first zoeal stage (df = 12, F = 236.62; P < 0.0001). The lowest average stage duration was recorded

for continuously fed *L. boggessi* and *L. seticaudata* larvae (2.0 ± 0.0 days) produced from fed zoea I (Table 4). Second zoeal stage duration was never longer than 4 days, with larvae either dying or molting to the third larval stage when reaching this period (Table 4). With the exception of continuously fed larvae, zoea II produced from starved zoea I always displayed significantly longer ($P < 0.0001$) larval duration periods than zoea II produced from fed zoea I (Table 4).

Table 4 – Average zoea II duration (days) (± standard deviation) of *Lysmata amboinensis*, *L. boggessi*, *L. debelius* and *L. seticaudata*, produced from fed or starved zoea I, under different initial starvation periods (24, 48 and 72 hours of starvation after hatching - S_{24}, S_{48} and S_{72}) and continuously fed and starved larvae (FC and SC, respectively) (n = 4 replicates of 60 larvae per each treatment, per each species) (zoea I fed – F; zoea I starved – S). Different superscript letters in the same row and different superscript numbers in the same column represent significant differences ($P < 0.05$). * Newly hatched larvae of these species could not molt to zoea II when continuously starved. ** Larvae died before molting to the next zoeal stage.

	L. amboinensis		*L. boggessi*		*L. debelius*		*L. seticaudata*	
	ZI F	ZI S	ZI F	ZI S	ZI F	ZI S	ZI F	ZI S
FC	$2.8 \pm 0.1^{a,1}$	*	$2.0 \pm 0.0^{b,1}$	$2.1 \pm 0.1^{b,1}$	$2.1 \pm 0.1^{b,1}$	*	$2.0 \pm 0.0^{b,1}$	$2.1 \pm 0.1^{b,1}$
S_{24}	$3.3 \pm 0.2^{a,2}$	*	$2.5 \pm 0.1^{b,2}$	$3.5 \pm 0.1^{ac,2}$	$2.5 \pm 0.2^{b,2}$	*	$2.4 \pm 0.1^{b,2}$	$3.7 \pm 0.4^{c,2}$
S_{48}	$4.0 \pm 0.0^{a,3}$	*	$2.7 \pm 0.1^{b,2}$	$4.0 \pm 0.0^{a,3}$	$3.1 \pm 0.3^{c,3}$	*	$2.7 \pm 0.1^{b,3}$	$4.0 \pm 0.0^{a,3}$
S_{72}	$4.0 \pm 0.0^{a,3}$	*	$2.8 \pm 0.1^{b,2}$	**	$4.0 \pm 0.0^{a,4}$	*	$3.2 \pm 0.3^{c,4}$	**
SC	$4.0 \pm 0.0^{a,3}$	*	$4.0 \pm 0.0^{a,3}$	**	$4.0 \pm 0.0^{a,4}$	*	$4.0 \pm 0.3^{a,5}$	**

4. Discussion

The present results show that the effect of early larval stages starvation within the genus *Lysmata* is not uniform. The most evident difference was the absence of FPL (see Anger 2001) in the two species from the Indo-Pacific reefs *L. amboinensis* and *L. debelius*. *Lysmata boggessi* and *L. seticaudata* display enhanced yolk reserves that allows them to successfully

molt to zoea II in the absence of food. This moderate food-independence is a highly advantageous feature for species inhabiting regions where larval preys patchiness may occur, and consequently, more or less prolonged periods of starvation can take place (Thessalou-Legaki et al., 1999). The vast majority of *Lysmata* species occurs in warm tropical waters, mainly coral reefs (Chace, 1997) and *L. seticaudata* is one of the few species occurring at higher latitudes (Udekem d'Acoz, 1999) and displaying a reduced number of larval stages (Calado et al., 2004). In this way, the existence of FPL could somehow be expected, since lower levels of food availability resulting from strong seasonality of plankton production at higher latitudes selects for abbreviated and/or lecitotrophic modes of development (Anger et al., 2004; Thatje et al., 2005). The occurrence of FPL in *L. boggessi* could not be so predictable, since this species is present in the warmer waters of the Gulf of Mexico (Rhyne and Lin, 2006). However, *L. boggessi* is a member of a cryptic "peppermint shrimp" species complex, which also includes *L. wurdemanni* (Gibbes, 1850), a species that occurs at higher latitudes and cooler waters (see Rhyne and Lin, 2006). Therefore, if the ancestral species of this "peppermint shrimp" complex displayed FPL, it is highly unlikely that such feature would not be present in the extant members of the complex. This assumption is supported by the lower natural constrains existing to develop lecitotrophic life strategies, when compared to those that could allow a lecitotrophic larval development to reverse back to non-lecitotrophic (Strathmann, 1978, 1985).

A remarkable feature recorded in the present work for all studied species was the ability of zoea II to advance to the next zoeal stage in the total absence of food. Although only a small proportion of individuals displayed such feature (commonly < 15% of starved larvae), this type of facultative secondary lecithotrophy (FSL) has never been previously recorded among caridean shrimps. Primary and secondary lecithotrophy mainly differ in the "fuel" used for food-independent larval development: an enhanced reproductive energetic investment by the female for the first type and accumulated plankton derived organic matter in the second

(Anger, 2001). Larval preys ingested in zoea I contribute to the build up of energetic reserves that are latter catabolized during the starvation periods in zoea II, allowing some larvae to molt to the third larval stage. Since no significant differences in survival were recorded among starved zoea II of *Lysmata* with FPL (*L. boggessi* and *L. seticaudata*) and those without such feature (*L. amboinensis* and *L. debelius*), it seems that energetic reserves present in newly hatched *L. boggessi* and *L. seticaudata* are totally depleted in starved larvae. These reserves are insufficient to allow starved zoea II to advance to the next larval stage and energy accumulated through larval prey's ingestion plays the crucial role. The true magnitude of FSL displayed by studied species may have been underestimated by the larval preys chosen for the present work (newly hatched *Artemia* nauplii). Despite being a widely used prey for larval decapods culture, *Artemia* nauplii have for long been recognized as unbalanced diet, particularly due to their essential fatty acids content (Monroig et al., 2006). Additionally, several studies have shown that even crustacean larvae "labeled" as carnivorous may also depend on the utilization of additional prey items, namely detritus, phytoplankton and protists (Sulkin and McKeen, 1999; Hinz et al., 2001; Perez and Sulkin, 2005; Schwamborn et al., 2006). In this way, it may be possible that in their natural environment a significantly higher percentage of larvae that have accumulated energetic reserves during the first zoeal stage, derived from a wider range of suitable larval preys, can endure starvation in zoea II and still advance to the next zoeal stage.

From a commercial perspective, the present results reinforce the need to provide suitable preys to newly hatched marine ornamental shrimps *Lysmata* immediately after hatching. This approach is vital for the successful culture of species not displaying FPL, namely the highly priced species *L. amboinensis* and *L. debelius*. Simões et al. (2002) have already highlighted the importance of minimizing starvation periods for newly hatched *L. debelius* larvae, in particular due to the timing between larval release and first larval feeding. For species displaying FPL, such as *L. boggessi* and *L. seticaudata*, starvation of newly hatched larvae

may not cause death but negatively affects them during the larval period, in particular through an increase in the duration of larval development, higher mortality at metamorphosis and asynchronous settlement (Calado et al., 2005a,b). Another important commercial aspect that may help minimize larval mortality induced by starvation is the improvement of parental ornamental shrimps broodstock diets used to promote gonad maturation. Poor quality maturation diets are known to produce poor larval batches and consequently poor yolk reserves at hatching. The main consequence of this deficiency is the occurrence of high larval mortality during the first days, even when suitable larval food is immediately provided after hatching. In marine ornamental shrimps, larvae originating from embryos spawned in the wild (yolk reserves have been mobilized from nutrients provided by natural parental diets) always display higher survival rates than those spawned in captivity. This success is probably due to quantitatively and/or qualitatively better yolk reserves of newly hatched larvae, which increase their tolerance to starvation periods and/or poor quality larval preys provided.

5. Conclusions

Currently, an informal division of genus *Lysmata*, based on the species sociobiology, is accepted: “crowd” species, with individuals living in high density aggregations, being nocturnal, generalized foragers, facultative fish cleaners and displaying a reddish "peppermint" stripes coloration; and “pairs” species, with individuals commonly living in pairs or trios, with diurnal habits, being specialized fish cleaners and displaying a bright colouration with stripes and spots of white colour strongly contrasting with a darker reddish background (Bauer, 2000). Curiously, in the present work, “crowd” species (*L. boggessi* and *L. seticaudata*) displayed FPL, while the larvae of both “pairs” species (*L. amboinensis* and *L. debelius*) did not present such ability. If future works verify this trend, the current phylogenetic theory that *Lysmata* “pairs” species are ancestral to “crowd” species (Bauer, 2006) can be supported. The rationale for this support would be that if FPL was an ancestral

feature it would be highly unlikely that it would have regressed to full planktotrophy in "pairs" species larvae (Strathmann, 1985).

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CAPÍTULO II

II. Importância da luz e morfologia larvar na resistência à inanição e capacidade de alimentação de larvas recém eclodidas de camarões ornamentais do género *Lysmata* (Decapoda: Hippolytidae).

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"Importance of light and larval morphology in stravation resistance and feeding ability of newly hatched marine ornamental shrimp *Lysmata* (Decapoda: Hippolytidae)" tendo como co-autores Ricardo Calado, Gisela Dionísio, Cátia Bartilotti, Cristóvão Nunes, Antonina dos Santos e Maria Teresa Dinis.

Importance of light and larval morphology in starvation resistance and feeding ability of newly hatched marine ornamental shrimps *Lysmata* spp. (Decapoda: Hippolytidae)

Ricardo Calado, Gisela Dionísio, Cátia Bartilotti, Cristóvão Nunes, Antonina dos Santos and Maria Teresa Dinis

Abstract

The present work evaluates the resistance of newly hatched *Lysmata amboinensis*, *L. ankeri*, *L. debelius* and *L. seticaudata* larvae to 24, 48, 72, 96 and 120 hours of starvation after hatching, as well as their ability to capture newly hatched *Artemia* nauplii and enriched metanauplii, under different light regimes (24, 12 and 0 hours of light). Additionally, it analyses the feeding and swimming behavior of newly hatched larvae and compares their morphological and biometrical features. *Lysmata ankeri* and *L. seticaudata* displayed higher resistance to starvation than *L. amboinensis* and *L. debelius*, molting to zoea II in the absence of food. Larvae starved for longer periods and kept in darkness displayed higher survival rates, probably due to a lower energy consumption induced by reduced swimming activity. Light regimes did not influence *Lysmata* ability to capture larval preys, reinforcing the idea that these larvae do not display true hunting behaviors, rather relying on chance encounters with dietary preys. All *Lysmata* larvae consumed similar levels of *Artemia* nauplii, while *L. ankeri* and *L. seticaudata* were able to consume a significantly higher amount of enriched metanauplii than *L. amboinensis* and *L. debelius*. Interspecific larval size variability, similar swimming ability and the inexistence of morphological features more specialized for prey capture in *L. ankeri* or *L. seticaudata* larvae exclude larval morphology and biometry as explanations for the different consumption rates recorded. The ability of *Lysmata* larvae to capture large dietary preys opens good perspectives for the use of inert diets at an early stage.

Key words: *Lysmata*, starvation resistance, larval feeding, larval morphology, light regimes, marine ornamental shrimps

1. Introduction

Larviculture of marine ornamental shrimps of genus *Lysmata* has been considered as the main bottleneck impairing commercial scale culture of these highly priced species (Calado et al., 2003a, b). Feeding of newly hatched larvae plays a crucial role in the success of *Lysmata* larviculture, since heavy mortality of early larval stages and asynchronous metamorphosis occurs when larvae are exposed to starvation after hatching (Simões et al., 2002; Calado et al., 2005a, b). In extreme situations, starved larvae may even reach a "point of no return". According to Anger and Dawirs (1981) this might be described as a threshold at which larvae, even when subsequently fed, are incapable of recovering from the nutritional stress imposed by early starvation, cannot resume their larval development and finally die. Exposure to starvation of newly hatched *Lysmata* larvae commonly occurs due to the timing of larval release by parental broodstock, which generally occurs during the night. Consequently, first larval feeding usually takes place several hours after hatching (Simões et al., 2002). Additionally, there is a generalized and erroneous idea that all newly hatched caridean shrimps larvae have the ability to molt from zoea I to zoea II in the absence of food. This ability is termed primary lecithotrophy and can be facultative if newly hatched larvae retain their ability to capture and ingest dietary preys (Anger, 2001). Although some newly hatched *Lysmata* larvae can indeed molt to the next zoeal stage under starvation (Zhang et al., 1998), the nutritional stress imposed is latter reflected in a prolonged larval development and by an increase in mortality at metamorphosis (Calado et al., 2005a).

Besides avoiding exposure to starvation, it is also highly important to provide suitable dietary preys to newly hatched larvae. Comprehensive studies on larval functional morphology and feeding behavior can play a vital role for the successful culture of larval decapod crustaceans,

namely by the valuable information provided on potential dietary prey characteristics (Crain, 1999; Cox and Johnston, 2003; Epelbaum and Borisov, 2006). However, there is very little information on larval morphology of the genus *Lysmata* (see Calado et al., 2004 for a review) and only Rhyne et al. (2001) have performed a preliminary analysis on the feeding kinematics of larval *Lysmata*. From a commercial perspective, it is highly important to ascertain if newly hatched larvae from different species of ornamental shrimps can accept similar food items, since species specific diets would certainly result in an increase in production costs.

In general, long photoperiod improves the performance of fish larvae, probably because of increased food availability (e.g. Boeuf and Le Bail, 1999; Fielder et al., 2002; Gimenez and Estevez, 2008). However, the effect of variable photoperiods in the larviculture of decapod crustaceans has commonly promoted species dependent effects, with some species being unaffected (Harvey and Epifanio, 1997) and others displaying deleterious effects when exposed to 0 or 24 h of light (Knowlton, 1974; Mikami and Greenwood, 1997). Although decapod crustacean larvae are not generally recognized as visual predators (e.g. Epelbaum and Borisov, 2006) it is possible that light may positively influence larval swimming activity, which may increase the chance of finding potential preys (Strathmann, 1987).

The study of larval feeding and swimming behavior is of paramount importance to correctly access the suitability of dietary preys. In fact, D'Abramo (2002) suggests that a correct evaluation of the acceptance of specific diet may only be properly evaluated through a detailed video analysis of the feeding behaviour of cultured larvae. The rationale for this approach is that a particular dietary item may have a suitable size and texture, as well as an adequate nutrient profile, but still not be accepted by cultured organisms.

In this way, the objectives of the present work were to ascertain the resistance of newly hatched larvae of *Lysmata amboinensis* (De Man, 1888), *L. ankeri* Rhyne and Lin, 2006, *L. debelius* Bruce, 1983 and *L. seticaudata* (Risso, 1816) to variable periods of starvation, as well as their ability to capture newly hatched *Artemia* nauplii and enriched metanauplii, under

the influence of different light regimes (24, 12 and 0 h of light). Additionally, the feeding and swimming behavior of newly hatched *Lysmata* larvae is also analyzed and their morphological and biometrical features are compared.

2. Materials and methods

2.1 Larval Production

Three breeding pairs each of *L. amboinensis*, *L. ankeri, L. debelius* and *L. seticaudata* in simultaneous hermaphrodite phase were kept in a maturation system described by Calado et al. (2007a). Salinity was maintained at 35 ± 1‰ and temperature was kept at 25 ± 1ºC through a heating/cooling system. Ammonia and nitrite were maintained below detectable levels, while nitrate and pH showed average values (± standard deviation, S.D.) of 3.0 (± 2.5) mgl^{-1} and 8.2 (± 0.1), respectively. Breeding pairs were fed with Marine Cuisine®, a commercial frozen diet for marine aquarium organisms produced by San Francisco Bay Brand® (Newark, CA, USA). The diet was provided on a dry-weight basis, with each breeding pair being fed 3 times per day (07.00, 14.00 and 21.00 h) in a combined total of 15% of each shrimp pair biomass.

In order to promote a similar quality between produced larvae of tested *Lysmata* species, the first three embryo batches produced after pairing were not considered for the present study, assuring that nutrients mobilized to developing gonads, and consequently to egg yolk reserves, were provided by the dietary items used in captivity.

The most active newly hatched larvae (the ones with pronounced positive phototactic responses, swimming near the actinic fluorescent light placed in the larval collection area of the broodstock aquariums described by Calado et al. (2007a)) were selected for morphological descriptions, starvation and feeding trials.

2.2 Larval morphology

Ten newly hatched larvae from each species were sampled, fixed and preserved with 4% formalin. Drawings and measurements of larval morphology were made with the aid of a *camera lucida* on a Wild M8 stereomicroscope, while setal observations and drawings were made using a Zeiss microscope also with a *camera lucida*. The preparation of slides with larval appendages was temporary. Larval description followed the method proposed by Clark et al. (1998) and setal and spine terminology is according to Calado et al. (2004). The long plumose setae on distal exopod segments are drawn truncated and setules from setae were omitted from drawings when necessary. Setal counts refer from proximal to distal sequence.

2.3 Larval biometry

Thirty newly hatched larvae from each *Lysmata* species were sampled and measured on Wild M8 stereomicroscope with a calibrated micrometer eyepiece. The following measurements were taken: Total Length (TL) - distance between tip of rostrum and posterior end of telson, Carapace Length (CL) - distance from tip of rostrum to posterior margin of carapace, Rostrum Length (RL) - distance from tip of rostrum to posterior margin of eye, Telson Length (TeL) - distance from what will be the anterior margin of telson in the next zoeal stage (since in zoea I the telson is fused with the 5th abdominal segment) to the posterior margin of median telson notch, Maxilliped Endopod Length (MxpEnL) (for the first, second and third pair of maxillipeds) - distance between anterior margin of first endopod segment and posterior tip of last endopod segment, and Maxilliped Exopod Length (MxpExL) (for the first, second and third pair of maxillipeds) - distance between anterior margin of first exopod segment and posterior tip of last exopod segment. The following ratios were calculated: CL/TL, RL/CL, RL/TL, TeL/TL, Mxp1EnL/TL, Mxp2EnL/TL, Mxp3EnL/TL, (Mxp1EnL/TL)+(Mxp2EnL/TL)+(Mxp3EnL/TL), Mxp1ExL/TL, Mxp2ExL/TL, Mxp3ExL/TL, and (Mxp1ExL/TL)+(Mxp2ExL/TL)+(Mxp3ExL/TL). See Figure 1 for an

overview on *Lysmata* larvae morphological features used in biometric analysis. The rationale for the selection of the morphological features described above was the direct or indirect influence that these characters have on the larval swimming and feeding performance of larval decapod crustaceans (see Crain, 1999; Epelbaum and Borisov, 2006).

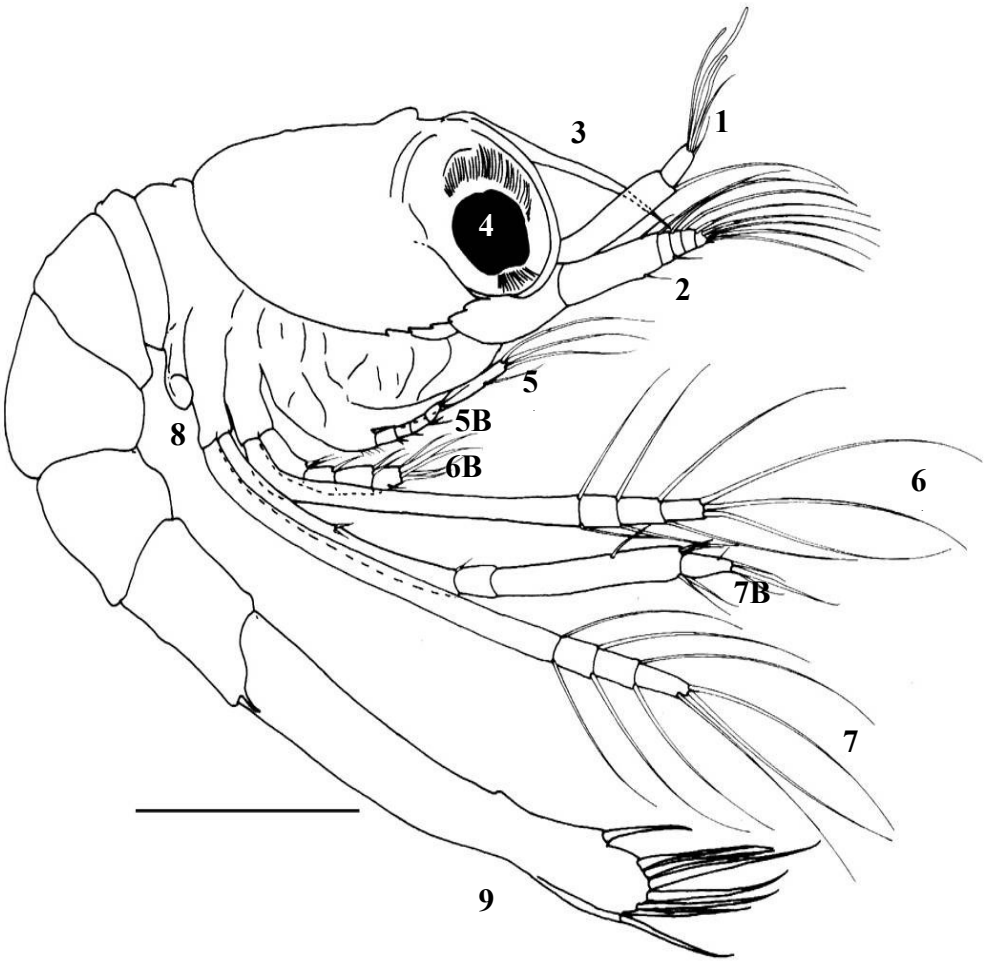

Figure 1. Lateral view of newly hatched larvae of *Lysmata amboinensis*: (1) Antennule with aesthetascs present apically in the outer flagellum; (2) Antenna; (3) Slender and pointed rostrum; (4) Compound sessile eyes fused with carapace; (5A) Exopod of first maxilliped; (5B) Endopod of first maxilliped; (6A) Exopod of second maxilliped; (6B) Endopod of second maxilliped; (7A) Exopod of third maxilliped; (7B) Endopod of third maxilliped; (8) Biramous bud of first pereiopod; (9) Telson fused with fifth abdominal segment. Scale bar: 500 μm.

2.4 Larval starvation trials

Starvation resistance of each *Lysmata* species newly hatched larvae was compared by randomly selecting newly hatched larvae, of at least three larval batches produced by shrimps from the same species, and by placing each one in starvation in small plastic containers (20ml each) under different light regimes (24, 12, and 0 hours of light), with the containers being illuminated from above with fluorescent white light, with an intensity of 18.5 μmol m^{-2} s^{-1} at the water surface. Groups of 300 larvae were divided in five equal groups of 60 individuals with larval survival being checked 24 h after hatching for group 1, 48 h after hatching for group 2, 72 h after hatching for group 3, 96 h after hatching for group 4 and 120 h after hatching for group 5 (5 groups of 60 larvae * 4 shrimp species * 3 light regimes * 5 starvation periods = 18000 larvae; 60 larvae per treatment * 5 replicates). No further starvation periods were tested, since a preliminary study revealed that newly hatched larvae of all tested species died when placed in starvation for 144 hours. Every day, the water from each larval container was 100% renewed with 1 μm filtered artificial seawater (Crystal Sea® salt manufactured by Marine Enterprises International® (Baltimore, MD, USA)), with temperature being kept at 25 ± 1ºC through a water bath connected to a heating/cooling system. Due to the large number of larvae used in the present study, and since not all studied species released their larvae simultaneously, it was impossible to perform all experimental trials at once. In this way, the different treatments and their respective replicates were performed in consecutive weeks, with the maximum number of larvae being manipulated in a single day never exceeding 3600 (the equivalent to 60 experimental treatments).

2.5 Larval feeding trials

Larval feeding ability of each *Lysmata* species was compared by randomly selecting 5 replicates of 50 newly hatched larvae, of at least three larval batches produced by shrimps from the same species, and by placing each larvae in small plastic containers (20ml each)

under the same light regimes described for larval starvation trials (24, 12 and 0 hours of light), and by providing them with the following larval preys: newly hatched *Artemia* nauplii (average size 450 ± 10 μm) and 24 h old metanauplii (average size average size 590 ± 10 μm) enriched for 24 h with *Cypthecodinium* spray dried biomass, at a density of 3 preys ml^{-1} (for both nauplii and metanauplii) (5 groups of 50 larvae * 4 shrimp species * 3 light regimes * 2 larval prey types = 6000 larvae; 50 larvae per treatment * 5 replicates). Larvae were allowed to feed for a 24 hour period, with remaining live and actively swimming larval preys being counted individually (one by one) under a binocular Wild M8 stereomicroscope. The variable used to access feeding was the total number of larval preys ingested after the 24 h period. In order to ensure a homogenized distribution of preys in the water column during the 24h experimental period, minimising the positive phototactic behavior of shrimp larvae and dietary preys, each 20 ml plastic containers was gently aerated at a rate of "one air bubble per second". Temperature was kept at 25 ± 1ºC through a water bath connected to a heating/cooling system. The larval feeding trials were not run simultaneously with those described in the previous section (larval starvation trials). During larval feeding experiments the maximum number of larvae being monitored on a single day was never superior to 240.

Table 1 - Morphological features of newly hatched *Lysmata amboinesis*, *L. ankeri*, *L. debelius* and *L. seticaudata* larvae.

Morphological Features	*Lysmata amboinensis* Indo-Pacific Present study	*Lysmata ankeri* Western Atlantic Present study	*Lysmata debelius* Indo-Pacific Present study	*Lysmata seticaudata* Eastern Atlantic Calado et al., 2004*
CARAPACE				
Rostrum	Slender & pointed (as long as antennul. ped.)	Slender & pointed (as long as antennul. ped.)	Slender and pointed (reaching half of antennul. ped.)	Slender & pointed (reaching 3/4 of antennul. ped.)
Eyes	Compound and sessile	Compound and sessile	Compound and sessile	Compound and sessile
ANTENNULE				
Peduncle segments/setal formula	1/1	1/1	1/1	1/1
Outer flagellum segments/setal formula	1/1+1+3 aesthetascs	1/1+1+3 aesthetascs	1/1+1+3 aesthetascs	1/1+4 aesthetascs
ANTENNA				
Protopod segments/setal formula	1/0	1/0	1/0	1/0
Endopod segments/setal formula	0/1+1	0/1+1	0/1+1	0/1+1
Scaphocerite segments/setal formula	5/2+1,1+1,1,1,4+1	5/2+1,1+1,1,1,4+1	5/2+1,1+1,1,1,4+1	5/2+1,1+1,1,1,4+1
MANDIBLE	Asymmetrical, no palp	Asymmetrical, no palp	Asymmetrical, no palp	Asymmetrical, no palp
MAXILLULE				
Coxal endite setae	7	6-7	7	7
Basial endite setae	5	5	5	5
Endopod segments/setal formula	0/2+3	0/2+1+3	0/2+3	0/2+3
MAXILLA				
Coxal endite setal formula	8+4	8-9+4	8-9+4	8+4
Basial endite setal formula	4+4	4+4	4+4	4+4
Endopod segments/setal formula	0/3+2+1+3	0/3+2+1+3	0/3+2+1+3	0/3+2+1+3
Scaphognathite setae	5	5	5	5
FIRST MAXILLIPED				
Coxal endite setae	5	5	5	5
Basial endite setae	12	12	12	12
Endopod segments/setal formula	4/3,1,2,1+3	4/3,1,2,1+3	4/3,1,2,1+3	4/3,1,2,3

Exopod segments/setal formula	0/1+3	0/1+3	0/1+3	0/1+3
SECOND MAXILLIPED				
Basial endite setal formulae	1+2+3	1+2+3	1+2+3	2+2
Endopod segments/setal formula	4/3,1,2,1+5	4/3,1,2,1+5	4/3,1,2,1+5	4/3,1,2,1+5
Exopod segments/setal formula	4/2,2,2,3	4/2,2,2,3	4/2,2,2,3	3/2,2,4
THIRD MAXILLIPED				
Basial endite setae	3	3	3	3
Endopod segments/setal formula	4/2+1,0,1+4,1+3	4/2+1,0,1+4,1+3	4/2+1,0,1+4,1+3	4/2+1,0,2+4,3
Exopod segments/setal formula	4/2,2,2,3	5/2,2,2,2,3	5/2,2,2,2,3	4/2,2,2,4
PEREIOPODS				
Buds present	P1biramous	P1 biramous	P1 biramous	P1 biramous; P5 uniramous
TELSON				
Posterior margin processes	7+7	7+7	7+7	7+7

2.6 Swimming and feeding behavior analysis

Zoeal swimming and feeding behavior was first recorded while larvae were in 20 l cylindrico-spherical larviculture tanks (adapted from those described by Calado et al. (2003c)). Thirty larvae from each *Lysmata* species were later randomly selected from the larviculture tanks and each one transferred to a 20 ml glass beaker. Swimming and feeding behavior were observed with the help of a binocular Wild M8 stereomicroscope and immediately noted by the observer for later interpretation. Behavioral observations were performed by larvae, with an average time of observation of 30 minutes per specimen. While swimming behaviour could basically be observed through the whole observation period, 30 minutes was the time necessary to monitor at least 3 different feeding events per larvae. The food types used for studying feeding behavior were the same used to quantify larval feeding (newly hatched *Artemia* nauplii and enriched metanauplii) and were offered to larvae at a density of 3 preys ml^{-1}. All behavioral observations were performed in the presence of light.

2.7 Data analysis

Each biometrical variable analyzed for newly hatched *Lysmata* spp. larvae was compared by a one-way analysis of variance ANOVA. Resistance to starvation and feeding ability were compared using a multi-way factorial analysis of variance MANOVA. The factors tested on each MANOVA were: 1)*Lysmata* species (4 levels), 2) light regime (3 levels), and 3) duration of starvation period (5 levels) for the resistance to starvation experiment; and 1) *Lysmata* species (4 levels), 2) light regime (3 levels), and 3) type of larval prey (2 levels) for the feeding ability experiment. Statistical analysis were performed using the software Statistica (version 6.0) and prior to analysis, assumptions were verified. Percentage data is known to have a significant departure from normality. In this way, prior to analysis, raw data were transformed using the arcsin (sqrt x) formula. Whenever significance was accepted, at $P < 0.05$, the Tukey multiple comparison test was used (Zar, 1999).

3. Results

3.1 Larval morphology

The morphological features of newly hatched *L. amboinensis*, *L. ankeri, L. debelius* and *L. seticaudata* larvae, with emphasis to appendages segmentation and setation, are described in Table 1. Figure 2 illustrates the morphological features of newly hatched *Lysmata* larvae analyzed in the present work. The peduncle of the antennule is unsegmented, exhibiting 1 long plumose seta terminally. Its outer flagellum is short with 1 plumodenticulate seta and 3 (or 4 in *L. seticaudata*) aesthetascs. The protopod of the antenna is unsegmented and the endopod displays 1 long plumose seta and 1 short spine apically. The scaphocerite is 5-segmented with 4 short segments distally, presenting 2 plumose setae on the outer side and 9 plumose setae on inner side, plus a simple small seta on apex. The pre-oral chamber is composed by the labrum and the lobes of the paragnaths, with the first displaying a densely setose area (with simple setae) on its lower margin. The oral chamber is bordered by

asymmetrical mandibles displaying their cutting edges perpendicularly aligned with the larvae saggital plane. The maxillules are flattened, with their coxal and basal endites being setose and toothed, exhibiting microtricha in the adjacent margins of the endites. The maxillae display bilobed coxal and basal endites with papposerrate setae, unsegmented endopodite and scaphognathite with plumose setae and microtricha on the endites margins. The last segment of the endopod of all maxillipeds show well developed papposerrate setae, with the second and third maxillipeds also exhibiting such setae on the inner margins of their endopods segments. The exopods of all maxillipeds display large plumose natatory setae. Although newly hatched *Lysmata* larvae displayed similar morphological features, some minor differences could be recorded among the different species. The most conspicuous difference was the presence of the first and fifth pereiopods (as a well developed biramous and a uniramous bud, respectively) on newly hatched *L. seticaudata*. In newly hatched larvae of remaining *Lysmata* species, the first pereiopod is present as a very small biramous bud and the fifth pereiopod is still absent. Zoea I of *L. seticaudata* displayed 4 aesthetascs on the outer flagellum of the antenulle, while the larvae of other *Lysmata* species only displayed 3 aesthetascs. The aesthetascs of *L. ankeri* displayed a unique appearance, compared to other *Lysmata* species, presenting a large bulge at their posterior end, rather than a typical finger-shape. Concerning the exopod of the second maxilliped, *L. seticaudata* only displayed 3 segments and 8 plumose setae, while all other studied species displayed 4 segments and 9 plumose setae. *Lysmata ankeri* and *L. debelius* displayed a total of 5 segments and 11 plumose setae on the exopod of the third maxilliped, while *L. amboinensis* and *L. seticaudata* displayed 4 segments and 9 and 10 plumose setae, respectively.

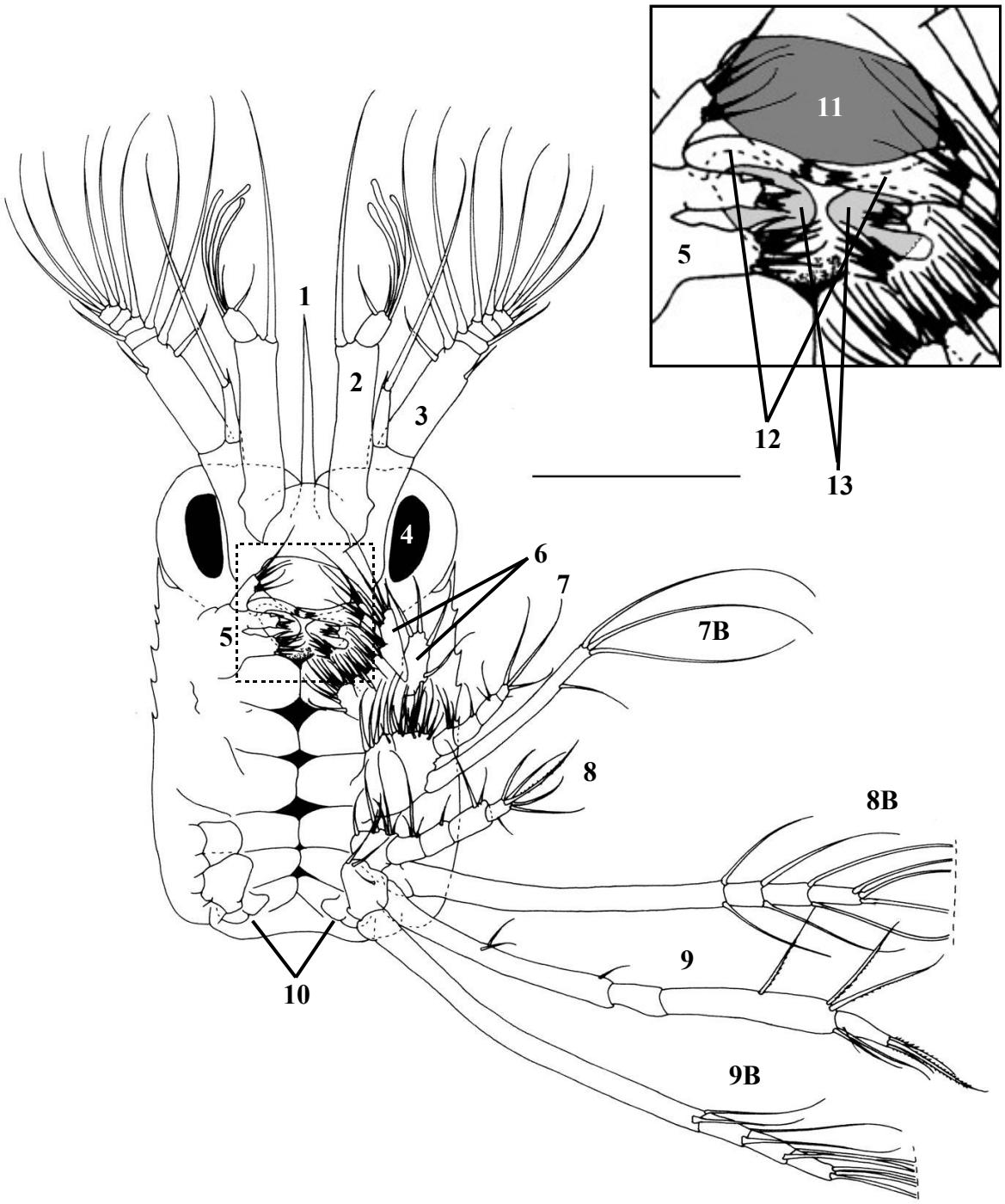

Figure 2. A - Ventral view of carapace region of newly hatched larvae of *Lysmata amboinensis* (thoracic appendages have been partially omitted on the left side); B - Magnification of the oral chamber: (1) Rostrum; (2) Antennule with aesthetascs present apically in the outer flagellum; (3) Antenna; (4) Compound sessile eyes fused with carapace; (5) Maxillule; (6) Maxilla; (7A) Endopod of first maxilliped; (7B) Exopod of first maxilliped; (8A) Endopod of second maxilliped; (8B) Exopod of second maxilliped; (9A) Endopod of third maxilliped; (9B) Exopod of third maxilliped; (10) Biramous bud of first pereiopod; (11) Labrum; (12) Mandibles; (13) Paragnaths. Scale bar: 500 μm.

3.2 Larval biometry

The biometric features recorded for newly hatched *L. amboinensis*, *L. ankeri, L. debelius* and *L. seticaudata* larvae are summarized in Table 2. The largest newly hatched larvae recorded were those of *L. seticaudata*, with an average TL of 3.17 ± 0.07 mm. The same species also displayed a significantly larger rostrum (P = 0.0022), while CL and TeL was not significantly different among studied larvae. Although newly hatched larvae of *Lysmata* displayed similar Mxp1EnL and Mxp2EnL, *L. seticaudata* displayed significantly smaller (P = 0.0041) Mxp3EnL. Concerning MxpExL, *L. seticaudata* larvae displayed the smallest sizes for all maxillipeds (Table 2). The ratios CL/TL, RL/TL and Te/TL was not significantly different for all studied species, although the ratio RL/CL was significantly (P < 0.0001) higher for newly hatched *L. seticaudata*. The analysis of the ratios MxpEnL/TL only revealed the existence of significant differences for the third maxilliped, with *L. seticaudata* displaying a significantly (P < 0.0001) lower ratio. *Lysmata seticaudata* larvae also displayed a significantly (P < 0.0001) lower (Mxp1En/TL)+(Mxp2En/TL)+(Mxp3En/TL) ratio. The ratios Mxp2ExL/TL, Mxp3ExL/TL and (Mxp1Ex/TL)+(Mxp2Ex/TL)+(Mxp3Ex/TL) were all significantly (P < 0.0001) lower in newly hatched *L. seticaudata* larvae. *Lysmata amboinensis* and *L. debelius* displayed the highest (Mxp1Ex/TL)+(Mxp2Ex/TL)+(Mxp3Ex/TL) ratios (Table 2).

Biometric Features	*L. amboinensis* Indo-Pacific	*L. ankeri* Western Atlantic	*L. debelius* Indo-Pacific	*L. seticaudata* Eastern Atlantic
TL (mm)	2.75 ± 0.05 [a]	2.97 ± 0.07 [b]	2.66 ± 0.08 [a]	3.17 ± 0,07 [b]
CL (mm)	0.93 ± 0.03 [a]	1.00 ± 0.04 [a]	0.89 ± 0.04 [a]	1.01 ± 0.02 [a]
RL (mm)	0.35 ± 0.04 [a]	0.31 ± 0.04 [a]	0.29 ± 0.02 [a]	0.46 ± 0.02 [b]
TeL (mm)	1.02 ± 0.02 [a]	1.04 ± 0.04 [a]	0.95 ± 0.05 [a]	1.08 ± 0.02 [a]
Mxp1EnL (mm)	0,28 ± 0.02 [a]	0,23 ± 0.02 [a]	0,23 ± 0.01 [a]	0,21 ± 0.02 [a]
Mxp2EnL (mm)	0,36 ± 0.03 [a]	0,39 ± 0.03 [a]	0,34 ± 0.03 [a]	0,34 ± 0.02 [a]
Mxp3EnL (mm)	1,26 ± 0.04 [a]	1,24 ± 0.04 [a]	1,39 ± 0.03 [a]	0,84 ± 0.02 [b]
Mxp1ExL (mm)	0,64 ± 0.02 [a]	0,49 ± 0.01 [b]	0,49 ± 0.01 [b]	0,48 ± 0.01 [b]
Mxp2ExL (mm)	0,97 ± 0.01 [a]	1,26 ± 0.04 [b]	1,09 ± 0.02 [ab]	0,82 ± 0.01 [c]
Mxp3ExL (mm)	1,19 ± 0.02 [a]	1,08 ± 0.02 [b]	1,16 ± 0.03 [ab]	0,68 ± 0.02 [c]
CL/TL	0.34 ± 0.02 [a]	0.34 ± 0.02 [a]	0.34 ± 0.02 [a]	0.32 ± 0.02 [a]
RL/CL	0.38 ± 0.03 [a]	0.31 ± 0.02 [a]	0.33 ± 0.02 [a]	0.46 ± 0.04 [b]
RL/TL	0,13 ± 0.02 [a]	0,10 ± 0.01 [a]	0,11 ± 0.02 [a]	0,15 ± 0.02 [a]
TeL/TL	0.37 ± 0.02 [a]	0.35 ± 0.03 [a]	0.36 ± 0.03 [a]	0.34 ± 0.01 [a]
Mxp1En/TL	0,10 ± 0.01 [a]	0,08 ± 0.01 [a]	0,08 ± 0.01 [a]	0,06 ± 0.01 [a]
Mxp2En/TL	0,13 ± 0.01 [a]	0,13 ± 0.02 [a]	0,13 ± 0.02 [a]	0,11 ± 0.02 [a]
Mxp3En/TL	0,46 ± 0.03 [ab]	0,42 ± 0.02 [a]	0,52 ± 0.03 [b]	0,26 ± 0.02 [c]
(Mxp1En/TL)+(Mxp2En/TL)+(Mxp3En/TL)	0,69 ± 0.03 [ab]	0,63 ± 0.03 [a]	0,73 ± 0.03 [b]	0,44 ± 0.02 [c]
Mxp1Ex/TL	0,23 ± 0.02 [a]	0,16 ± 0.01 [a]	0,19 ± 0.02 [a]	0,15 ± 0.02 [a]
Mxp2Ex/TL	0,35 ± 0.02 [a]	0,43 ± 0.02 [a]	0,41 ± 0.03 [a]	0,26 ± 0.01 [b]
Mxp3Ex/TL	0,43 ± 0.03 [a]	0,36 ± 0.02 [a]	0,44 ± 0.03 [a]	0,21 ± 0.01 [b]
(Mxp1Ex/TL)+(Mxp2Ex/TL)+(Mxp3Ex/TL)	1,02 ± 0.02 [a]	0,95 ± 0.03 [a]	1,03 ± 0.04 [a]	0,62 ± 0.03 [b]

Table 2 - Average biometrical features (± standard deviation) of newly hatched *Lysmata amboinesis*, *L. ankeri*, *L. debelius* and *L. seticaudata* larvae (n = 30 larvae per species). TL - Total Length, CL - Carapace Length, RL - Rostrum Length, TeL - Telson Length, MxpEnL - Maxilliped Endopod Length, and MxpExL - Maxilliped Exopod Length. Different superscript letters in the same row represent significant differences ($P < 0.05$).

3.3 Larval starvation trials

Average survivals of newly hatched *Lysmata* larvae starved for 24, 48, 72, 96 and 120 hours and placed under different light regimes are summarized in Table 3. There was no significant interaction between shrimp species * light regime * starvation period ($df = 24$, $F = 0.902$; $p = 0.5995$). Significant interactions were detected between shrimp species* light regime ($df = 6$, $F = 4.18$; $p = 0.0007$), shrimp species * starvation period ($df = 12$, $F = 5.96$; $p < 0.0001$) and light regime * starvation period ($df = 8$, $F = 2.74$; $p = 0.0081$). In general, *L. ankeri* and *L. seticaudata* larvae displayed highest survival values during different starvation periods and under different light regimes (Table 3). Although no clear relation could be established between the different light regimes and starvation periods lasting 24 or 48 hours, larvae from all *Lysmata* species starved for 72 hours or more always displayed higher survivals in the absence of light (Table 3). When starved for periods longer than 48 hours, less than 50% of newly hatched *L. debelius* and *L. amboinensis* larvae survived. While all *L. ankeri* and *L. seticaudata* surviving larvae were able to molt to zoea II after 48 hours in the absence of food, no starved *L. debelius* or *L. amboinensis* were able to advance to the next larval stage. Zoea II of *L. ankeri* and *L. seticaudata* that remained in starvation in trials lasting 72, 96 and 120 hours were unable to molt to zoea III.

Table 3 - Average survival (%) (± standard deviation) of newly hatched *Lysmata amboinesis*, *L. ankeri*, *L. debelius* and *L. seticaudata* larvae, starved for 24, 48, 72, 96 and 120 hours and placed under different light regimes (24, 12 and 0 hours of light) (n = 5 groups of 60 larvae per each species, per each starvation period and per each light regime). Different superscript letters in the same row and different superscript numbers in the same column represent significant differences (P < 0.05). L - hours of light.

	Starvation Periods				
	24 h	48 h	72 h	96 h	120 h
L. amboinensis L24	66.7 ± 6.7 ^{a,1}	53.3 ± 13.3 ^{ab,1}	13.3 ± 11.5 ^{bc,1}	2.2 ± 0.8 ^{c,1}	0.0 ± 0.0 ^{c,1}
L. amboinensis L12	80.0 ± 13.3 ^{a,1}	66.7 ± 13.3 ^{ab,1}	24.4 ± 13.9 ^{bc,1}	2.2 ± 0.8 ^{cd,1}	0.0 ± 0.0 ^{d,1}
L. amboinensis L 0	66.7 ± 29.1 ^{a,1}	55.6 ± 19.2 ^{a,1}	40.0 ± 13.3 ^{a,12}	2.2 ± 0.8 ^{b,1}	0.0 ± 0.0 ^{b,1}
L. ankeri L 24	91.1 ± 7.7 ^{a,1}	71.1 ± 23.4 ^{a,1}	48.9 ±22.3 ^{a,123}	2.2 ± 0.8 ^{b,1}	0.0 ± 0.0 ^{b,1}
L. ankeri L 12	100.0 ± 0.0 ^{a,1}	95.6 ± 3.8 ^{a,1}	73.3 ± 29.1 ^{a,23}	20.0±11.5 ^{b,12}	0.0 ± 0.0 ^{c,1}
L. ankeri L 0	100.0 ± 0.0 ^{a,1}	97.8 ± 3.8 ^{a,1}	82.2 ± 19.2 ^{a,23}	57.8 ±23.6 ^{ab,2}	33.3 ±10.6 ^{b,2}
L. debelius L 24	73.3 ± 17.6 ^{a,1}	62.2 ± 21.5 ^{a,1}	6.7 ± 4.7 ^{b,1}	0.0 ± 0.0 ^{b,1}	0.0 ± 0.0 ^{b,1}
L. debelius L 12	82.2 ± 10.2 ^{a,1}	60.0 ± 20.6 ^{a,1}	4.4 ± 2.7 ^{b,1}	0.0 ± 0.0 ^{b,1}	0.0 ± 0.0 ^{b,1}
L. debelius L 0	68.9 ± 15.4 ^{a,1}	57.8 ± 16.9 ^{a,1}	22.2 ± 7.8 ^{b,1}	0.0 ± 0.0 ^{c,1}	0.0 ± 0.0 ^{c,1}
L. seticaudata L 24	100.0 ± 0.0 ^{a,1}	100.0 ± 0.0 ^{a,1}	60.0 ± 26.7 ^{a,23}	6.7 ± 2.0 ^{b,1}	0.0 ± 0.0 ^{b,1}
L. seticaudata L 12	97.8 ± 3.8 ^{a,1}	97.8 ± 3.8 ^{a,1}	93.3 ± 6.7 ^{a,3}	42.2 ± 19.2 ^{b,2}	0.0 ± 0.0 ^{c,1}
L. seticaudata L 0	97.8 ± 3.8 ^{a,1}	97.8 ± 3.8 ^{a,1}	97.8 ± 3.8 ^{a,3}	66.7 ±20.0 ^{ab,2}	22.2 ±15.4 ^{b,2}

3.4 Larval feeding trials

The average consumption of newly hatched *Artemia* nauplii and 24 h old metanauplii by newly hatched *Lysmata* larvae under different light regimes are shown in Table 4. There was no significant interaction between shrimp species * light regime * larval prey type (df = 6, F = 0.75; p = 0.6109). Significant interactions between shrimp species * light regime (df = 6, F = 3.97; p = 0.0006) and shrimp species * larval prey type (df = 3, F = 25.83; p < 0.0001) were recorded, while no significant interaction was detected between light regime * larval prey type (df = 2, F = 0.03; p = 0.9684). No significant effect was detected in the average consumption of larval preys promoted by the type of prey (df = 1, F = 1.17; p = 0.2789).

Lysmata amboinensis and *L. debelius* zoea exposed to different light regimes always ingested a higher number of newly hatched *Artemia* nauplii than metanauplii, while *L. seticaudata* ingested a similar percentage of both larval preys. The lowest consumption rates were recorded for *L amboinensis* and *L. debelius* zoea preying on enriched metanauplii in the absence of light (37.3 ±19.3% and 38.5 ± 19.8%, respectively), while the highest consumption rate was recorded for *L. ankeri* fed enriched metanauplii under 24 or 0 hours of light (76.2 ± 12.2% and 76.2 ± 10.8%, respectively). *Lysmata ankeri* newly hatched larvae were the only ones to ingest a higher number of *Artemia* metanauplii than newly hatched nauplii, with this difference being significant ($p < 0.0001$) when larvae were exposed to 24 hours of light 0 hours of light (Table 4).

Table 4 - Average consumption (%) (± standard deviation) of newly hatched *Artemia* nauplii and 24 h old metanauplii by newly hatched *Lysmata amboinesis*, *L. ankeri*, *L. debelius* and *L. seticaudata* larvae under different light regimes (24, 12 and 0 hours of light) (n = 250 larvae per each species, per each type of prey and per each light regime). Different superscript letters in the same row and different superscript numbers in the same column represent significant differences ($P < 0.05$). L - hours of light.

	L 12		L 24		L 0	
	Nauplii	Metanauplii	Nauplii	Metanauplii	Nauplii	Metanauplii
L.amboinensis	52.8 ± 23.1 ^{a,1}	46.7 ± 24.3 ^{ab,1}	52.2 ± 22.4 ^{a,1}	41.2 ± 19.3 ^{ab,1}	48.6 ± 18.7 ^{ab,1}	37.3 ±19.3 ^{b,1}
L. ankeri	62.4 ± 19.3 ^{abcd,1}	74.1 ± 12.4 ^{ac,2}	60.5±20.0 ^{ad,12}	76.2 ± 12.2 ^{c,2}	57.9 ± 12.9 ^{d,12}	76.2 ±10.8 ^{c,2}
L. debelius	58.1 ± 18.5 ^{ab,1}	47.6 ± 18.7 ^{ac,1}	62.7 ±20.6 ^{b,12}	57.9 ± 17.4 ^{ab,12}	50.5 ± 18.8 ^{abc,12}	38.5 ±19.8 ^{c,1}
L.seticaudata	62.8 ± 21.6 ^{a,1}	61.8 ± 18.6 ^{a,2}	73.0 ±14.3 ^{a,2}	69.9 ± 17.8 ^{a,12}	63.2 ± 18.5 ^{a,2}	62.6 ±27.1 ^{a,2}

3.5 Swimming and feeding behavior analysis

Newly hatched *Lysmata* larvae commonly swim telson first and "upside-down". Swimming is achieved through the "stroke" movements of the exopods of the maxillipeds, which bear long plumose setae. The rapid beating motion of this larval structure creates water currents towards the anterior end of the larva and sends the larvae on a thrusting movement, with its telson leading. The movement of the maxillipeds exopods occurs in succession and not in a synchronous pattern. The telson plays the major role on the adjustment of swimming direction, with the endopods of the maxillipeds always being direct towards the anterior part of the larvae and not seeming to play any role on larval swimming.

Stationary and swimming *Lysmata* larvae were observed capturing and ingesting newly hatched *Artemia* nauplii, as well as enriched metanauplii. Apparently, only preys approaching the thoracic region of the larvae, near the exopods, triggered a predatory response. The endopods of the maxillipeds were always the first larval appendages involved in the process of prey capture. Swimming *Artemia* nauplii or enriched metanauplii were "speared" by the papposerrate setae of the terminal segment of the third maxilliped endopod, although the second maxilliped endopod could sometimes also be used. After "spearing" the prey, *Artemia* nauplii or enriched metanauplii were passed from the larval maxillipeds to the maxilla, to the maxillule and finally to the mandibles. The paragnaths seem to help in preventing food loss from the mouth area, while the labrum function during this process could not be clearly identified. Before ingestion, preys were commonly oriented by the maxillule and mandible in a way that their longitudinal body axis would be perpendicular with that of the larvae, with *Artemia* nauplii or enriched metanauplii being ingested head or tail first. Apparently the telson never seemed to play any role in the prey capture/manipulation process. The average duration of a feeding event, from prey capture to full ingestion, ranged between 52 and 145 seconds. Preys were recorded escaping from capturing larvae only when they were struggling to break away from the "spearing" setae of the maxillipeds endopods. Concerning prey ingestion, the

entire body of *Artemia* nauplii or enriched metanauplii was commonly ingested by *Lysmata* newly hatched larvae, although several times prey were only nibbled, generally on the cephalic appendages and outer portion of the abdomen, and were later discarded. Only rarely (3 observations) would a larva ingesting a prey try to capture another *Artemia* nauplii or enriched metanauplii. The anecdotal reports of *Lysmata* larvae thrusting forward towards dietary preys or actively chasing a larval prey were not confirmed during the present work. Whenever an *Artemia* nauplii or enriched metanauplii touched the larvae carapace, including the rostrum, *Lysmata* larvae strongly swam away in an apparent avoidance response.

4. Discussion

Despite the remarkable morphological and behavioral similarity exhibited by newly hatched *L. amboinensis*, *L. ankeri, L. debelius* and *L. seticaudata* larvae, their ability to capture dietary preys displayed significant interspecific variability. In general, larval ability to effectively capture dietary preys can be influenced by the following aspects: 1) larval natatory ability, 2) dietary preys' predator avoidance behaviors, 3) morphological adaptations of larval appendages to capture and manipulate preys, 4) prey detection mechanisms, 5) prey density, and 6) specific prey functional responses. According to the present morphometric comparison of the larval appendages employed by newly hatched *Lysmata* to swim (namely their maxilliped exopods), it appears that all studied species display similar natatory abilities. In this way, the higher number of preys captured by *L. ankeri* or *L. seticaudata* does not seem to be a consequence of a superior natatory ability. Despite their larger size, *L. seticaudata* larvae exhibited the lowest (Mxp1Ex/TL)+(Mxp2Ex/TL)+(Mxp3Ex/TL) ratio, which can probably indicate a reduced natatory ability when compared to other *Lysmata* larvae. Nonetheless, newly hatched *L. seticaudata* were more efficient capturing preys than other larvae with higher (Mxp1Ex/TL)+(Mxp2Ex/TL)+(Mxp3Ex/TL) ratios (e.g. *L. amboinensis* and *L. debelius*). Gonor and Gonor (1973) and Epelbaum and Borisov (2006) have highlighted that

despite zoea being good swimmers, true hunting behaviors (involving prey chasing) are not displayed and larvae entirely rely on chance encounters with dietary preys. This type of feeding behavior has been termed by Berkes (1975) as "encounter feeding". In the present work, the only predator avoidance behavior recorded for newly hatched *Artemia* nauplii and enriched metanauplii was a more vigorous beating of the cephalic appendages, namely on metanauplii, but only after being "speared" by the setae of the maxillipeds endopods . Therefore, the inferior ability displayed by *L. amboinensis* and *L. debelius* to capture larval preys could be explained if these species larvae could not firmly grasp captured prey. However, the morphological analysis does not support this theory, since larval appendages setation was highly similar between all tested species and *L. amboinensis* and *L. debelius* displayed (Mxp1EnL/TL)+(Mxp2EnL/TL)+(Mxp3EnL/TL) ratios similar to that of *L. ankeri*, the most efficient species capturing enriched metanauplii. The inexistence of morphological features more specialized for prey capture in *L. ankeri* or *L. seticaudata* larvae excludes larval morphology as an explanation for the different prey consumptions recorded. As previously referred, *Lysmata* larvae exhibit an "encounter feeding" behavior, meaning that despite having highly developed eyes, they do not play any special role during prey capture. In zoeal stages, the antennules and the antennas are the main structures involved in the mechanical and chemical perception of preys (Anger, 2001, 2006). Aesthetascs are well known specialized structures located in the antennules of larval decapods, being responsible for chemoreception (Laverack, 1988). Although *L. ankeri* newly hatched larvae displayed different shaped aesthetascs, *L. seticaudata* displayed an extra aesthetasc in the antennule and given that *Lysmata* zoea do not "track and chase" their prey, it does not look like that these larval features would be responsible for the prey capture results recorded. Nonetheless, it is possible that *L. ankeri* and *L. seticaudata* may be able to detect their preys more readily when these are within the range of their maxillipeds endopods.

Since morphological and biometrical features seem to be insufficient to explain the variable

prey capture abilities recorded for newly hatched *Lysmata* larvae, it is possible that different species display different prey preferences. Several studies have already suggested that larval decapods exhibit prey preference due to selective feeding, instead of differences in encounter rates and/or capture efficiency (e.g. Harvey & Epifanio, 1997; Narciso & Morais, 2001; Harvey & Morrier, 2003; Baylon et al., 2004). This type of larval behavior may maximize energetic intake (Harvey and Epifanio, 1997), which can indicate that different newly hatched *Lysmata* larvae display different energetic requirements. Additionally, it may also be possible that larvae exposed to variable periods of starvation may shift their prey preference, in order to balance the energetic budget associated with prey capture and ingestion. Curiously, the larvae hatching with higher energetic reserves, the ones displaying facultative primary lecithotrophy (being able to molt from zoea I to zoea II using internal energetic reserves in the absence of food) (*L. ankeri* and *L. seticaudata*), were also the ones which seemed to have the highest energetic requirements (higher number of larval preys ingested). It is interesting to point that the larvae of *L. amboinensis* and *L. debelius* displayed a higher tolerance to starvation periods in the present study than that reported by Calado et al. (2007b), on which newly hatched larvae of these species could not endure more than 24 hours of starvation. Additionally, a recent study by Cunha et al. (2008) monitoring oxygen consumption in *L. amboinensis* larvae fed or starved during the first 24 h after hatching indicated that food ingestion does seems to be crucial during this period. These apparently contradictory results are probably a consequence of variable broodstock quality and/or suitability of maturation diets employed in both studies, reinforcing the need of future studies to clarify the role that energetic maternal investment in yolk reserves may play in shrimp larval quality.

The apparent dichotomy on larval behavior recorded in the present work may reflect the informal phylogenetic structure of the genus *Lysmata* proposed by Bauer (2006): “crowd” species (*L. ankeri* and *L. seticaudata*) *vs*. “pairs” species (*L. amboinensis* and *L. debelius*). This preliminary division suggests that “pairs” species are ancestral to “crowd” species, with

the first ones evolving in oligotrophic environments. If so, “pairs” species larvae may also had to endure low larval preys abundance, a selective pressure which probably favored lower larval energetic requirements.

Larviculture trials evaluating the effect of different light regimes have reported species dependent results. Minagawa (1994) reported lower prey consumption rates for newly hatched larvae of the red frog crab *Ranina ranina* kept in darkness than when kept with 24 hours of light. However, Harvey and Epifanio (1997) did not record any significant effect of light regimes on food intake displayed by newly hatched larvae of the common mud crab *Panopeus herbstii*. Unlike fish larvae, which are visual feeders (Blaxter, 1986), decapod crustaceans larvae do not use sight to locate and capture preys (Epelbaum and Borisov, 2006), with light probably playing a minor role than in fish larviculture (Boeuf and Le Bail, 1999). However, light may promote a more intense swimming activity, and consequently, affect prey ingestion indirectly by promoting more “chance encounters” with potential preys (Strathmann, 1987). According to the present results, there seems to exist no benefit on exposing newly hatched *Lysmata* larvae to “extreme” photoperiods (24 h darkness or 24 h light), since larval feeding rates are not significantly influenced. Additionally, other studies have already reported the occurrence of deleterious effects on larvae raised either in total darkness or with 24 h of light (Knowlton, 1974; Mikami and Greenwood, 1997).

The higher survival of *Lysmata* larvae starved for longer periods and kept in darkness may be explained by a decrease in swimming activity, and consequently on lower energy expenditure by these larvae, when compared to those exposed to 24 or 12 h of light. If suitable larval preys are not available to be immediately provided to newly hatched *Lysmata*, the deleterious effects of starvation may be minimized by keeping these larvae in total darkness until adequate food can be provided.

5. Conclusion

Newly hatched *Lysmata* morphology and biometry does not seem to provide reliable clues on larval prey size suitability, since this feature appears to be species-specific. A more reliable indicator appears to be the existence of facultative primary lecithotrophy, with larvae displaying this ability apparently being able to capture larger preys (e.g. *Artemia* metanauplii). A comparison between the energetic content of different prey items, along with an analysis on potential shifts on prey preferences promoted by variable starvation periods, may certainly help to clarify some of the patterns presented in this study and help to support or refute the "crowd" vs. "pair" species hypothesis. By providing enriched larval preys immediately after hatching, nutritional deficiencies that seem to induce delays and/or mortality at metamorphosis may be avoided. However, the fact that larvae sometimes only nibbled the cephalic appendages of *Artemia* metanauplii may decrease the efficiency of nutritional boosting provided by *Artemia* enrichments. At least for newly hatched larvae, the manipulation of light regimes is not a viable option to increase the ingestion of larval preys. The ability of *Lysmata* larvae to capture large preys (e.g. 590 ± 10 μm *Artemia* metanauplii) opens good perspectives for the use of inert diets at an early stage. Rhyne and Lin (2004) have already showed the great potential the use of inert diets may have for the culture of marine ornamental shrimps *Lysmata*. However, when testing inert larval diets light regimes may play a key role, since the positively phototactic newly hatched larvae commonly concentrate near the water surface (Mikami and Greenwood, 1997) and have limited access to sinking food particles in the bottom of the culture tank.

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Discussão

O sucesso no cultivo de crustáceos decápodes está dependente do período de exposição à inanição, dos diferentes estádios de desenvolvimento larvar e da espécie parental (Calado et al., 2007). No entanto, o conhecimento referente ao comportamento alimentar e às necessidades nutricionais das larvas é ainda predominantemente empírico (Barros & Valenti, 2003). Para uma melhor compreensão das necessidades inerentes ao cultivo de camarões ornamentais, a fim de evitar a mortalidade e atrasos na metamorfose, é importante conhecer o tipo de alimento adequado a cada estádio de desenvolvimento larvar, sendo este conhecimento particularmente relevante para os primeiros estádios de zoés.

I. Resistência à inanição de larvas recém eclodidas de camarões ornamentais do género *Lysmata.*

A imposição de períodos de inanição a larvas recém-eclodidas de camarões do género *Lysmata* permite aferir qual a resistência das diferentes espécies a este tipo de *stress* nutricional extremo. Os trabalhos realizados anteriormente, referentes a esta temática (Anger & Dawirs, 1981; Dawirs, 1984; Olson & Olson, 1989; Anger, 1995; Gimenez & Anger, 2005; Anger *et al.,* 2007) relatam uma grande variabilidade na capacidade das larvas de crustáceos decápodes ultrapassarem períodos de exposição à inanição permanentes e/ou intermitentes. A tolerância à inanição está relacionada com o estádio ontogénico, o investimento parental na produção de embriões e ainda as condições fisiológicas do animal no momento da captura e durante a sua manutenção em cativeiro (Böer *et al.,* 2006).

Com este trabalho foi possível verificar que a tolerância à inanição de larvas recém-eclodidas é variável no género *Lysmata.* Observa-se ainda a ausência de lecitotrofia primária facultativa (LPF) nas duas espécies que habitam os recifes do Indo-Pacífico, *L. amboinensis* e *L. debelius* quando sujeitas a inanição. Os resultados obtidos neste trabalho sugerem que as

larvas de crustáceos decápodes oriundas de águas tropicais apresentam reservas vitelinas insuficientes para a sua sobrevivência quando expostas a períodos de inanição. Por outro lado, as espécies *L. boggessi* e *L. seticaudata* possuem reservas endógenas que lhes permitem transitar do estádio de zoé I para zoé II na ausência de alimento.

Grande parte das espécies presentes no género *Lysmata,* encontram-se distribuídas em regiões de águas tropicais e subtropicais (Chace, 1997). A espécie *L. seticaudata* é uma das poucas que habita em regiões a maiores latitudes (Udekem d'Acoz, 1999), possuindo o menor número de estádios larvares até agora encontrado dentro do género (Calado *et al.,* 2004). O desenvolvimento abreviado da fase larvar, traduzido num menor número de estádios larvares em *L. seticaudata*, poderá ser uma adaptação à limitação do alimento no ambiente natural, permitindo o sincronismo com os picos de produção primária, já verificado para outros crustáceos decápodes (Thessalou-Legaki *et al.,* 1999; Thatje *et al.,* 2004. A sociobiologia das espécies de *Lysmata* foi estudada por Bauer (2006) e dividida em duas categorias: as espécies que vivem em grupos e habitam águas temperadas e sub-tropicais, e as espécies que coabitam em pares nas regiões de águas tropicais. Bauer (2006) remete para o facto das espécies que coabitam em grupos como *L. seticaudata e L. boggessi* (com menor número de estádios larvares), terem evoluído a partir dos ancestrais que viviam aos pares nas regiões de águas mais quentes (*L. amboinensis* e *L. debelius*). Por outro lado, as espécies que apresentam maior independência do alimento são aquelas que evoluíram possivelmente dos ancestrais de águas mais quentes, adpatando a estratégia lecitotrófica (Strathmann, 1978,1985). Uma vez que a disponibilidade de alimento planctónico é a maior força selectiva que ocorre em latitudes elevadas (Thatje *et al.,* 2004), os modos de desenvolvimento independentes de alimento são favoravelmente seleccionados (Anger *et al.,* 2004; Thatje *et al.,* 2005; Thessalou-Legaki *et al.,* 1999).

Os períodos de inanição impostos a larvas recém-eclodidas de *L. seticaudata e L. boggessi* não afectaram a sobrevivência e a duração do primeiro estádio, contudo, promovem

um desenvolvimento mais lento das larvas no estádio de zoé II (tabela 1 e 2, capítulo I). Contrariamente ao verificado para o primeiro estádio de zoé, a disponibilidade do alimento torna-se importante para o desenvolvimento das larvas de *L. seticaudata* e *L. boggessi* no estádio de zoé II. Embora, a ausência de alimento não induza mortalidade no estádio inicial de desenvolvimento larvar, devido ao elevado potencial endotrófico destes organismos (Emlet, 1986), a longo prazo os períodos de inanição promovem o aumento da duração do desenvolvimento larvar, um assentamento tardio e assíncrono, traduzindo-se muitas vezes em elevadas mortalidades durante a metamorfose (Calado *et al.,* 2005 Calado & Narciso, 2005).

A resistência à inanição, ou baixa "vulnerabilidade nutricional" (Sulkin, 1978), demonstrada pelas larvas de *L. boggessi* não era expectável, uma vez que esta espécie faz parte do complexo específico *wurdermanni* presente nas águas quentes do Golfo do México (Rhyne & Lin, 2004).

De acordo com os trabalhos de Strathmann (1978, 1985), se os ancestrais do complexo específico *wurdermanni* possuíssem LPF, seria pouco provável que esta característica não fosse evidenciada nos restantes membros do complexo. Evolutivamente, é mais simples manter a estratégia lecitotrófica do que reverter para um desenvolvimento larvar não lecitotrófico (Strathmann, 1978,1985).

Em geral, as larvas no estádio de zoé I apresentam grandes quantidades de vitelo (Gimenez, 2002). No entanto, quando o alimento se encontra disponível, a larva acumula reservas energéticas adicionais, tal como registado por Anger (1995) para o caso das larvas de *Sesarma curacaoense*. Os zoés II de *L. seticaudata* e *L. boggessi*, quando submetidos a períodos de inanição de 24h e 48h, demonstram um desenvolvimento larvar mais longo (S24, S48; Tabela 4, Artigo 1), comparativamente às larvas que não são expostas à inanição. As larvas no estádio de zoé II, resultantes de zoé I não alimentadas, não resistem a períodos de inanição superiores a 48 h. O atraso no desenvolvimento larvar poderá ser interpretado como um mecanismo de mobilização da energia entre dois processos geralmente síncronos, mas

independentes: o desenvolvimento morfológico e o crescimento.

Os resultados obtidos no presente estudo estão de acordo com a hipótese formulada por Anger (1995): as larvas que apresentam grandes quantidades de reservas vitelinas, submetidas a períodos de ausência de alimento, podem recorrer a uma alteração na estratégia de mobilização de energia. Em vez de canalizada para a morfogénese, grande parte da energia é utilizada para acelerar o crescimento. Assim, as larvas continuam a crescer, mas não se desenvolvem, e consequentemente, podem ser observados fénomenos de "mark time moulting". Segundo a definição de Gore, (1985), este fenómeno traduz-se numa sequência de mudas realizadas em determinado estádio larvar onde se podem observar poucas alterações morfológicas, sendo a mais conspícua o aumento do tamanho larvar (Calado *et al.*, 2003b).

As presas ingeridas em zoé I contribuem para o armazenamento das reservas energéticas que serão posteriormente catabolizadas durante eventuais períodos de inanição em zoé II, permitindo às larvas transitar, em algumas situações, para o estádio de zoé III. Deste modo, verificou-se a ocorrência de lecitotrofia secundária facultativa (LSF) nas larvas de diferentes espécies de *Lysmata* abordadas no presente trabalho. Este comportamento alimentar não tinha sido ainda registado para crustáceos da infra-ordem *Caridea*. Todas as espécies em estudo apresentaram a capacidade de transitar de zoé II para zoé III na ausência de alimento. Contudo, apenas alguns indivíduos apresentaram esta característica (< 15% das larvas em inanição), revelando a existência de variabilidade intra-específica.

Uma vez que não foram observadas diferenças significativas na sobrevivência entre larvas no estádio de zoé II com LPF (*L. seticaudata* e *L. boggessi*) e larvas que não apresentam esta característica (*L. amboinensis* e *L. debelius*), infere-se que as reservas energéticas das larvas recém-eclodidas de *L. seticaudata* e *L. boggessi,* são totalmente catabolizadas quando as larvas se encontram em inanição (Tabela 3, Capítulo I). As reservas resultantes do investimento parental são insuficientes para permitir a muda de zoé II para o estádio seguinte, sendo de extrema importância a ingestão de alimento no primeiro estádio para fazer face a

futuros períodos de inanição.

A principal diferença entre lecitotrofia primária e secundária difere na matéria utilizada para o desenvolvimento larvar independente de alimento. No primeiro caso, a energia catabolizada pelas larvas resulta da degradação do vitelo presente no embrião, resultando este do investimento parental. No segundo caso, ocorre a degradação de compostos resultantes da acumulação de alimento ingerido do plâncton (Anger, 2001).

Os resultados obtidos neste estudo mostram-se contraditórios com os de Cunha *et al.,* (2008). Estes autores, demonstram que as larvas recém-eclodidas de *L. amboinensis* submetidas a inanição durante 24 h, sofreram um decréscimo nas reservas energéticas (triacilglicerol), comparativamente às larvas alimentadas, sugerindo LPF. Verificaram ainda que o oxigénio catabolizado por larvas em inanição se manteve constante, tanto em larvas alimentadas como em inanição, indicando que o alimento não é crucial durante este período.

Uma vez que a capacidade de lecitotrofia está dependente do investimento energético parental na reprodução (Anger, 2001), e na qualidade do alimento fornecido aos progenitores, as diferenças registadas nos resultados poderão de certa forma estar relacionadas com o investimento parental. A fim de garantir que apenas os nutrientes fornecidos pela dieta são mobilizados para a maturação das larvas de *L. amboinensis,* parece pouco provável, uma vez que estas larvas não sobrevivem a períodos superiores a 24 h de inanição e principalmente porque este estudo não demonstra a transição das larvas do estádio zoé I para zoé II na ausência de alimento.

No presente trabalho, as larvas desta espécie alimentaram-se logo após a eclosão, mas na ausência de alimento não conseguiram realizar a muda para o estádio seguinte, mostrando dependência do alimento. Segundo Gimenez (2002), a biomassa limitada nas primeiras fases larvares, parece aumentar os efeitos negativos do período de inanição na duração do desenvolvimento larvar. Considerando as larvas *L. amboinensis* mais vulneráveis ao *stress* nutricional que as espécies *L. seticaudata, L. boggessi* ou mesmo *L. ankeri,* parece pouco

provável que estas larvas consigam transpôr os estádios seguintes.

A utilização de um regime pouco diversificado e menos rico energéticamente (nauplius de *Artemia*), pode ter influenciado a capacidade de exibir LSF para as espécies estudadas. As larvas de crustáceos possuem hábitos alimentares carnívoros, contudo podem alimentar-se de detritos, fitoplâncton e protistas (Jones *et al.*, 1997).

Em ambiente natural, a disponibilidade de presas é maior e mais diversificada, possibilitando às larvas maior resistência à ausência de alimento. Assim, os resultados deste trabalho poderão apenas elucidar o comportamento alimentar das larvas em cativeiro, visto a dieta fornecida ser muito diferente da capturada em ambiente natural.

Espécies que não possuem LPF, como *L. amboinensis* e *L. debelius,* necessitam do fornecimento de alimento a curto prazo, a fim de minimizar os efeitos deletérios dos períodos de inanição. Simões *et al.,* (2002) já tinha verificado este facto, focando como crucial o tempo entre a eclosão das larvas e a primeira alimentação. Para larvas recém-eclodidas que possuem LPF, como *L. boggessi* e *L. seticaudata,* a inanição poderá não causar mortalidade, mas produzir efeitos negativos ao longo do desenvolvimento da larva (Calado *et al.,* 2005, Calado & Narciso, 2005).

O facto deste estudo ter sido realizado em ambiente controlado, e sendo as espécies estudadas animais poiquilotérmicos, faz com que os resultados obtidos estejam condicionados pela temperatura imposta em cativeiro.

Um dos aspectos mais importantes, no que diz respeito à produção comercial destes organismos, é o fornecimento de dietas de maturação adequadas aos pares de reprodutores mantidos em cativeiro. Este procedimeno poderá minimizar a mortalidade larvar induzida por eventuais períodos de inanição fortuita (ex. desfasamento temporal entre a eclosão larvar e o fornecimento de alimento, indisponibilidade de alimento larvar devido a problemas com a cadeia trófica acesssória) uma vez que as reservas vitelinas das larvas aquando da eclosão serão significativamente superiores.

II. Importância da luz e morfologia larvar na resistência à inanição e capacidade de alimentação de larvas recém-eclodidas de camarões ornamentais do género *Lysmata.*

As espécies *L. seticaudata, L. amboinensis, L. ankeri* e *L. debelius* apresentam diversas semelhanças nos comportamentos associados à captura e manipulação do alimento e na morfologia dos apêndices envolvidos nestas funções. No entanto, a capacidade de predação das larvas analisadas neste estudo mostram a existência de variações interespecíficas.

A captura de alimento por parte das larvas é influenciada pela sua capacidade de natação, eventuais adaptações morfológicas, pela facilidade de manipulação dos organismos capturados, mecanismos de detecção e comportamentos de fuga das presas. De acordo com os resultados obtidos, e através dos comportamentos observados nas larvas recém eclodidas, a capacidade de natação não exerce um papel preponderante na captura das presas testadas (nauplius e metanauplius de *Artemia*).

Tal como observado em outras espécies de crustáceos decápodes (Epelbaum & Borisov, 2006), as larvas de *Lysmata* nadam através do batimento activo dos exópodes dos maxilípedes e pereiópodes, utilizando o telson para orientar a direcção do seu movimento. A corrente gerada pelos exópodes, cria um movimento de água na zona lateral e ventral da superfície da larva. Quando a larva nada activamente, as presas de menores dimensões que passam perto da região torácica e abdominal são arrastadas pela corrente criada na região ventral da larva. O alimento é assim, colocado na proximidade dos endópodes dos maxílipedes, sendo estes os primeiros apêndices envolvidos na abordagem à presa e no processo de captura. O telson, nesta fase, promove uma força propulsora que auxilia os endópodes dos maxílipedes na captura de alimento. As larvas de *Lysmata* dispõem na zona terminal dos endópodes dos maxílipedes sedas paposerradas que possibilitam uma captura mais eficiente dos nauplius e metanauplius de *Artemia* enriquecidos. Estas sedas assemelham-se a pequenos arpões que ao

arpoarem a presas dificilmente a libertam.

No que diz respeito às características biométricas abordadas, a espécie *L. seticaudata,* possui o *ratio* mais baixo entre o tamanho dos exópodes dos diferentes maxílipedes e o comprimento total, assim como entre o telson e o comprimento larvar. Este aspecto poderá indicar que as larvas desta espécie possuem menor capacidade natatória do que as larvas das restantes espécies estudadas. Contudo, as larvas de *L. seticaudata* apresentam um maior número de presas capturadas em relação às espécies *L. amboinensis e L. debelius*. Deste modo, as diferenças morfológicas e biométricas detectadas não permitem explicar a variabildade registada na capacidade predatória das larvas. Estes resultados são concordantes com o trabalho de Epelbaum & Borisov (2006). Estes autores encontraram em zoés de *Paralithodes camtschticus* (Tilesius, 1815) uma habilidade natatória muito desenvolvida, embora estes organismos não persigam as presas e a captura das mesmas esteja dependente da probabilidade de encontro. Este tipo de comportamento foi descrito por Berkes (1975) como "encontro casual" entre predador e presa.

No presente trabalho, a única estratégia de fuga observada por parte das presas (nauplius recém-eclodidos de *Artemia* e metanauplius enriquecidos) foi o aumento do batimento dos apêndices cefálicos, depois destes entrarem em contacto com as sedas existentes nos endópodes dos maxilípedes das larvas.

A menor capacidade predatória observada em *L. debelius* e *L. amboinensis,* seria facilmente explicada se as larvas destas espécies não conseguissem agarrar as presas de forma eficaz. Contudo, as análises morfológicas não suportam esta hipótese, uma vez que o tipo e número de sedas presentes nos apêndices larvares envolvidos na captura e manipulação das presas é semelhante entre as diferentes espécies estudadas. A elevada ingestão de presas por larvas de *L. ankeri* e de *L. seticaudata,* também não poderá ser explicada pela análise comparativa da sua morfologia, uma vez que não se observaram quaisquer estruturas especializadas para este fim. Como já foi referido anteriormente, as larvas de *Lysmata* não exibem comportamentos de

predação activos, parecendo encontrar as suas presas casualmente. Este comportamento sugere que as estruturas visuais não possuem um papel determinante para a captura de alimento.

Para as larvas de crustáceos decápodes, as antenulas e as antenas são as estruturas responsáveis pela percepção mecânica e química do meio envolvente e as primeiras intervenientes na abordagem da larva a potenciais presas (Anger, 2001, 2006). As principais estruturas especializadas na quimiorecepção são os astetascos localizados nas antenulas (Laverack, 1988). As observações realizadas demonstram que os astetascos de larvas recém-eclodidas de *L. ankeri* possuem uma forma diferente dos observados nas diferentes espécies estudadas, apresentando as larvas de *L. seticaudata* mais um aestetasco na antenula do que as restantes.

No que diz respeito ao fotoperíodo, os estudos realizados em algumas espécies de crustáceos, incluindo *Carcinus maenas* (Linnaeus, 1758) (Dawirs, 1984) e *Thenus orientalis* (Lund, 1793) (Mikami & Greenwood, 1997), mostram que os regimes de luz influenciam os ritmos endógenos responsáveis pela regulação da *ecdysis* e, consequentemente, nas respostas de crescimento. Minawaga (1994), observou que larvas de *Ranina ranina* (Linnaeus, 1758) consomem menos presas quando mantidas em escuridão, do que se mantidas em 24 horas de luz. Contrariamente, Harvey & Epifanio (1997), não observaram efeitos significativos na capacidade de alimentação de larvas de *Panopeus herbstii* H. Milnee-Edwards 1834, após exposição a diferentes regimes de luz.

Contudo, os efeitos do fotoperíodo no comportamento (natatório e predatório) e no desenvolvimento larvar variam interespecificamente. Uma vez que a maior parte das larvas de crustáceos decápodes apresenta fototáxia positiva, é possível que a actividade natatória aumente, durante as fases luminosas, e consequentemente a probabilidade de encontro com as presas aumente também. Por outro lado, a presença de luz constante ao promover o aumento da actividade natatória das larvas, reduz as hipóteses de sobrevivência uma vez que estas

permanecem constantemente activas (elevado consumo energético). O *stress* imposto pelo excesso de exposição à luz pode ser exclusivamente responsável pelo declínio na ingestão de presas, tal como verificado para as larvas de *Jasus edwardsii* (Hutton, 1875) (Bermudes & Ritar, 2008). Aparentemente, a ocorrência de um fotoperíodo de 12 h de luz optimiza a performance alimentar e consequentemente o crescimento, sugerindo que a transição entre o dia e a noite adquiram um papel fundamental nesta fase do desenvolvimento larvar.

Contrariamente ao observado para as larvas de peixe, as larvas de crustáceos decápodes não utilizam a visão para localizar e capturar as presas (Epelbaum & Borisov, 2006), tendo a luz um papel menos relevante. Contudo, a luz poderá promover um aumento da actividade natatória (Sulkin, 1984) e, consequentemente promover uma maior captura de presas resultante de um maior número de encontros casuais (Strathmann, 1987). A luz, serve ainda de estímulo à orientação dos animais no plano vertical (Lagerspetz & Vainio 2006), embora as respostas larvares possam ser alteradas pela temperatura, luz e salinidade (Sulkin, 1984). De acordo com os resultados obtidos, não existe qualquer benefício na exposição de larvas de *Lysmata* a fotoperíodos extremos (24 e 0 h de luz), uma vez que a taxa de ingestão de presas não apresenta diferenças significativas.

Observou-se ainda, que as larvas de *Lysmata* que se encontravam em inanição por períodos de tempo superiores, mas privadas de luz, exibiram sobrevivências superiores aos restantes tratamentos. Em situações ocasionais em que seja impossivel evitar a exposição das larvas à inanição (ex. indisponibilidade de alimento devido a problemas na cadeia de cultivo acessório), os efeitos deletérios provocados por estes períodos de ausência de alimento poderão ser mitigados através da manutenção das larvas na escuridão.

Considerações finais

A tolerância das larvas a diferentes períodos de ausência de alimento é variável entre as diferentes espécies de *Lysmata* estudadas. As larvas que apresentam LPF, possuem reservas energéticas que lhes permitem transitar do estádio I para o estádio II na ausência de alimento.

A aparente dicotomia (espécies gregárias e espécies que habitam em pares) observada no comportamento larvar no género *Lysmata* poderá confirmar a relação filogenética anteriormente proposta por Bauer (2006). A divisão deste género baseia-se na ancestralidade das espécies que vivem em pares (*L. amboinensis e L. debelius*) relativemente às espécies que vivem em grupos (*L. seticaudata, L. ankerii*), tendo as primeiras evoluindo a partir de um ambiente oligotrófico. Assim, as larvas eclodidas em ambientes oligotróficos teriam de sobreviver à baixa abundância de presas alimentares. Através da influencia exercida pela pressão selectiva, as larvas com baixos requisitos energéticos podem ter sido inicialmente favorecidas. Será importante ainda referir que possam ter ocorrido adaptações à tolerância de inanição nas espécies que vivem em grupos, proporcionando-lhes maior flexibilidade na ausência ou presença de alimento. Os modos de desenvolvimento larvar existentes dentro do género *Lysmata* são mais complexos e menos generalizados do que seria esperado. Estudos futuros, que possam de certa forma integrar os conceitos de alimentação e composição bioquímica destas espécies, parecem ser importantes para confirmar ou refutar a teoria proposta por Bauer (2006).

Este trabalho, verificou a ausência de lecitotrofia primária facultativa nas espécies de recife de coral, e ainda a passagem do estádio de zoé II na ausência de alimento para zoé III, tanto nas espécies que coabitam em pares, nas regiões de águas quentes, como em grupos, nas regiões de latitudes mais elevadas. Adicionalmente, a existência de lecitotrofia secundária deveria ser investigada em outros carídeos, especialmente os que apresentam períodos de desenvolvimento mais longos, permitindo assim verificar a plasticidade trófica destes organismos. O conhecimento referente ao comportamento alimentar, necessidades

nutricionais e a resistência à inanição contínua ou intermitente de cada espécie, em cada estádio de desenvolvimento, torna-se crucial para o sucesso do cultivo destas espécies.

Uma vez que os caracteres morfológicos e biométricos foram insuficientes para explicar a variabilidade no número de presas capturadas pelas larvas recém-eclodidas, é possível que as várias espécies de *Lysmata* estudadas possuam preferências diferentes no que diz respeito às presas fornecidas. A selecção de presas exibida pelas larvas de decápodes é referida em diversos estudos como alternativa às diferenças encontradas na eficiência de captura e na probabilidade de encontro entre a larva e a presa (Harvey & Epifanio, 1997; Narciso & Morais, 2001; Harvey & Morrier, 2003; Baylon *et al.,* 2004). Este tipo de comportamento poderá trazer vantagens na maximização de energia ingerida pelas larvas (Harvey & Epifanio, 1997), e de certa forma promover diferenças interespecíficas ao nível dos requisitos energéticos.

A utilização de ferramentas moleculares, tem-se mostrado particularmente efectiva na identificação das presas capturadas pelas larvas, mostrando assim a grande variabilidade de alimento capturado em cada um dos estádios de desenvolvimento (Suzuki, 2007). O estudo da inanição nas diferentes fases do desenvolvimento dos crustáceos decápodes poderá servir de modelo para a compreensão das alterações moleculares e enzimáticas que ocorrem durante o processo de crescimento. A abordagem de ensaios enzimáticos para a determinação das variações ontogénicas ao longo do desenvolvimento larvar parece ser determinante para a optimização dos cultivos, podendo ser um indicador dos nutrientes a fornecer.

Como referido anteriormente, as presas vivas fornecidas neste trabalho (nauplius e metanauplius de *Artemia*) poderão de certa forma ter influenciado os resultados obtidos, pelo que seria interessante estudar os comportamentos alimentares destas larvas na presença de presas existentes no plancton.

Em conclusão, os comportamentos alimentares, a resistência à inanição e a

plasticidade nutricional (nomeadamente a existência de LSF) observados nestas espécies de habitats tão distintos, sugerem que outros crustáceos decápodes possam também evidenciar estas características. Estaremos a ignorar este facto?

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ANEXOS

ANEXO I

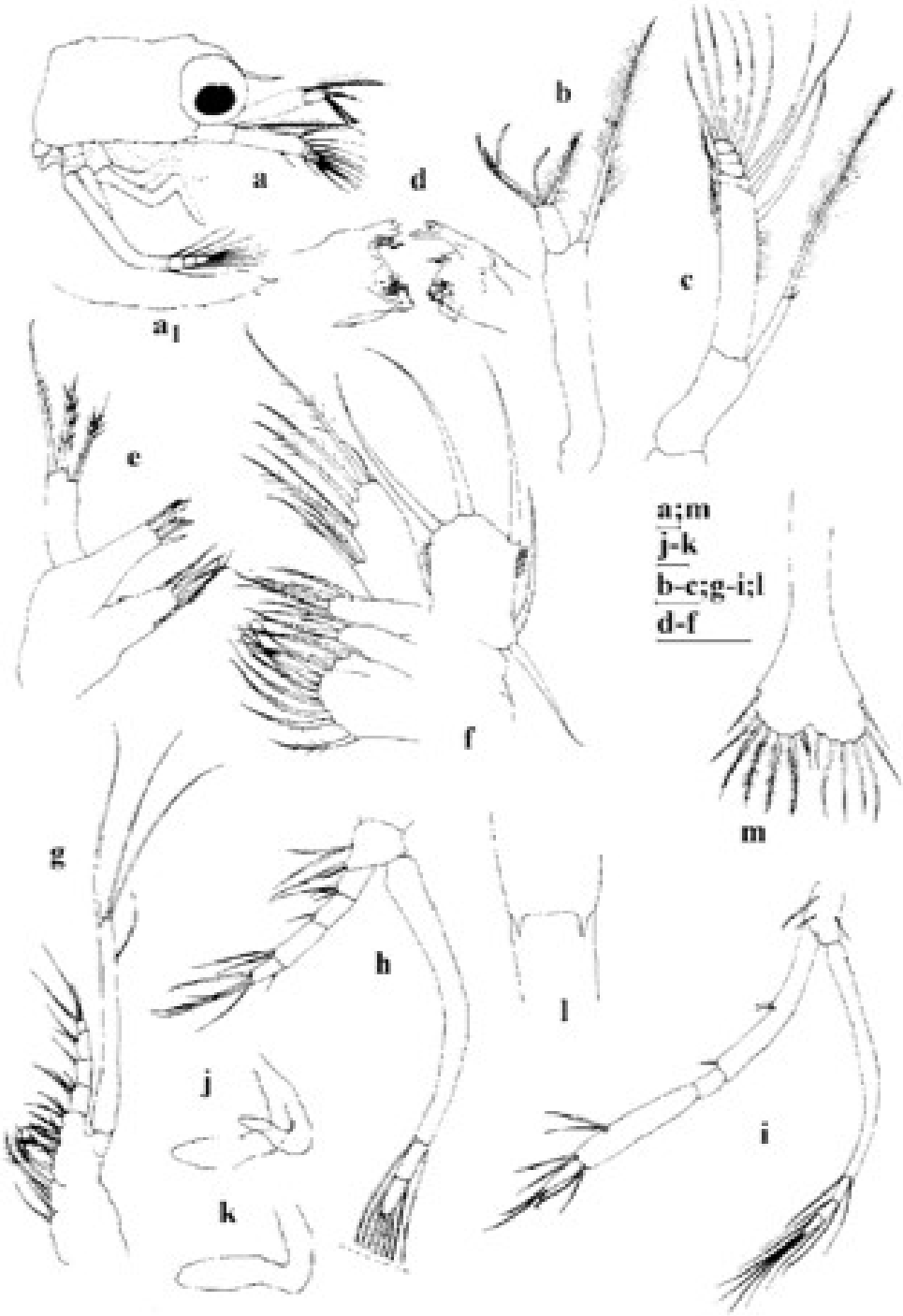

Figura 1. *Lysmata seticaudata.* **Primeira zoé:** (a) carapaça, vista lateral; (a_1) margem ventral da carapaça; (b) antênula; (c) antena; (d) mandíbula; (e) maxilula; (f) maxíla; (g) primeiro maxilípede; (h) segundo maxilípede; (i) terceiro maxilípede; (j) primeiro pereiopode; (k) quinto pereiopode, (l) detalhe do quinto sómito abdominal; (m) telson. Escala: 100 µm (Adaptado de Calado*et al.*, 2004).

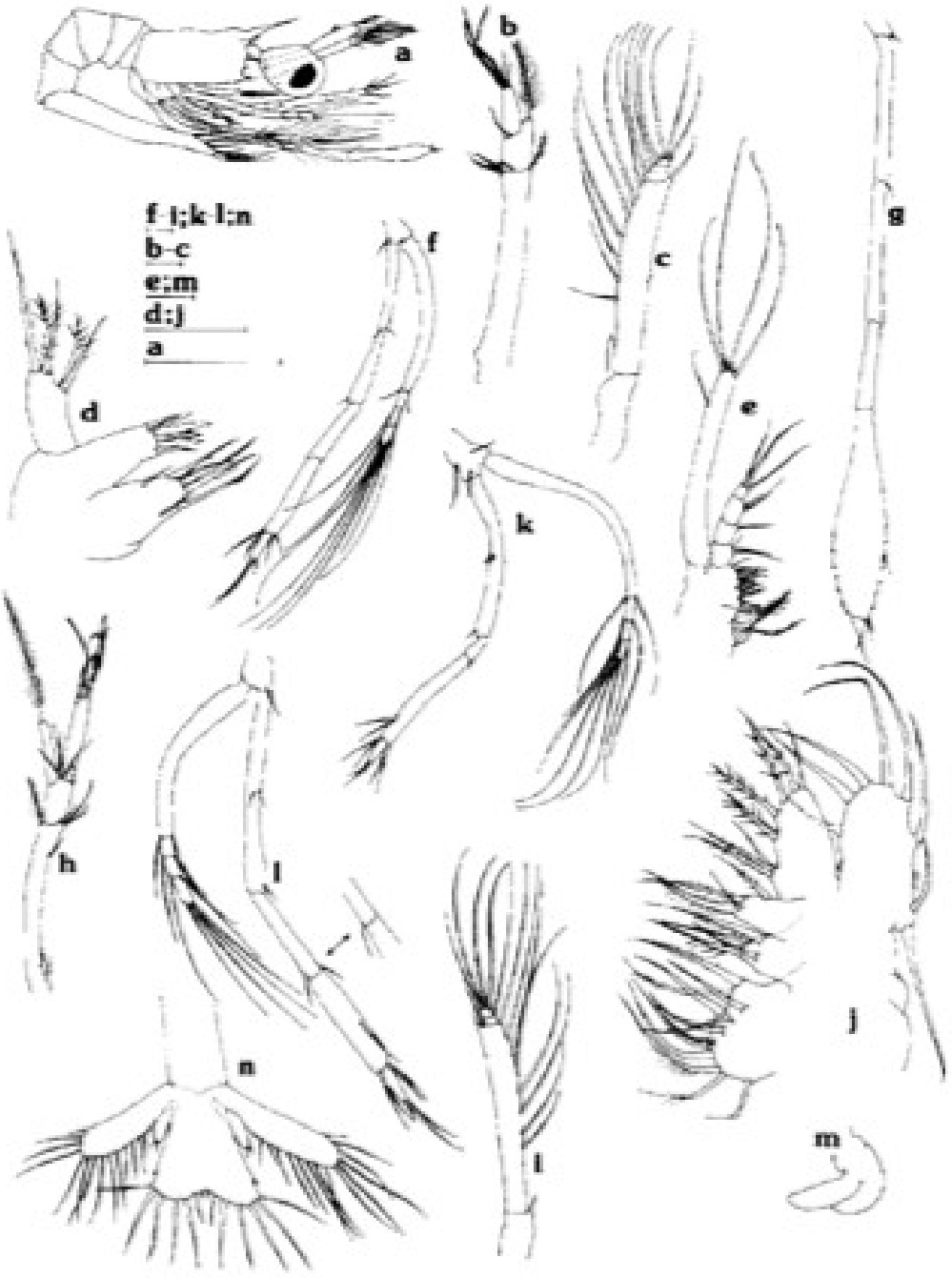

Figura 2. *Lysmata seticaudata.* **Segunda zoé:** (a) vista lateral; (b) antênula; (c) antena; (d) maxilula; (e) primeiro maxilípede; (f) primeiro pereíopode; (g) quinto pereíopode; **Terceira zoea**: (h) antênula; (i) antena (j) maxilula; (k) terceiro maxilípede (l) primeiro pereíopode; (l_1) detalhe do segundo segmento do endópode do primeiro pereíopode mostrando apenas a presença de uma seta; (m) segundo pereiopode; (n) telson e urópodes. Escala: 1000 μm (Adaptado de Calado*et al.*, 2004)**.**

ANEXO II

POSTER

Este poster foi apresentado no congresso internacional **"**The Crustacean Society Mid-Year Meeting" realizado em Comquimbo, Chile de 14 a 17 de Outubro do ano 2007, com o título: **"Importance of light and larval morphology in stravation resistance and feeding ability of newly hatched marine ornamental shrimp *Lysmata* (Decapoda: Hippolytidae)"** tendo como co-autores Gisela Dionísio, Cátia Bartilotti, Cristóvão Nunes, Antonina dos Santos, Maria Teresa Dinis e Ricardo Calado.

Importance of light and larval morphology in starvation resistence and feeding ability of newly hatched marine ornamental shrimps *Lysmata* spp. (Decapoda: Hippolytidae)

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Introduction

Feeding of newly hatched larvae plays a crucial role in the success of *Lysmata* larviculture, since heavy mortality of early larval stages and asynchronous metamorphosis occurs when larvae are exposed to starvation after hatching (Simões et al., 2002; Calado et al., 2005a, b).
In extreme cases, starved larvae may even reach a "point of no return". According to Anger and Dawirs (1981), this might be described as a threshold at which, even when subsequently fed, larvae are incapable to recover from the nutritional stress early imposed by starvation, can not resume their larval development and finally die.
Besides avoiding exposure to starvation, it is also important to provide suitable dietary preys to newly hatched larvae.

Objectives

❑ Ascertain the resistance of newly hatched larvae of *Lysmata amboinensis* (De Man, 1888), *L. ankeri* Rhyne and Lin, 2006, *L. debelius* Bruce, 1983 and *L. seticaudata* (Risso, 1816) to variable periods of starvation, and investigate their ability to capture different newly hatched *Artemia* nauplii and enriched metanauplii under the influence of different light regimes (24, 12 and 0 h of light).

❑ Analyse the feeding and swimming behaviour of newly hatched larvae of each *Lysmata* species referred above and compares their morphological and biometrical features.

Material and Methods

Six specimens of *L. amboinensis, L. ankeri, L. debelius* and *L. seticaudata* in simultaneous hermaphrodite sexual fase were used to form three breeding pairs for each species. The individuals were kept in a maturation system described by Calado et al. (2007). The most active newly hatched larvae were selected for morphological descriptions, starvation and feeding trials.

Measurements used to morphological and biometrical descriptions, were made using a *camera lucida* both on a Zeiss microscope binocular and in Wild M8 stereomicroscope with a calibrated micrometer eyepiece, respectively.

Larval feeding ability was compared by randomly selecting newly hatched larvae and placing each larvae in small plastic container under the same light regimes described above and providing newly hatched *Artemia* nauplii (450± 10 µm) and metanauplii (590±10 µm) enriched for 24 h.

Newly hatched larvae were selected and placed each one in starvation in small plastic containers under different light regimes (24, 12 and 0 h of light). Larval survival was checked 24, 48, 72, 96 and 120 h after hatching.

Swimming and feeding behaviour were observed with the help of a binocular Wild M8 stereomicroscope and immediately noted by the observer for interpretation.

Results

Larval morphology

Table 1 – Main differences observed in morphological features of newly hatched *L. amboinensis, L. ankeri, L. debelius* and *L. seticaudata* larvae. Abbreviations: pl- plumose setae, pp- pappose setae, ps- paposerrate, aes- aesthetascs, subt- subterminally, t- terminally, unseg- unsegmented. * Present study; ** Calado et al., 2004: setal terminology according with Garm, 2004.

Morphological Features	*L. amboinensis* Indo-Pacific*	*L. ankeri* Western Atlantic*	*L. debelius* Indo-Pacific*	*L. seticaudata* Eastern Atlantic**
ANTENNULE				
Outer flagellum: segment/setae	Unseg./1 short pl+1long pp+ 3aes	Unseg./1short pl+1long pp+3aes	Unseg./1short pl+1long pp+3aes	Unseg./1 short pl+4aes
MAXILLA				
Coxal endite	8+4 ps	8-9+4 ps	8-9+4 ps	8+4 ps
FIRST MAXILLIPED				
Exopod: segments/ setae	Unseg. / 1shorter subt+ 3long t pl	Unseg./ 1shorter subt+ 3long t pl	Unseg./ 1shorter subt+ 3long t pl	Unseg./1subt+3 t pl
SECOND MAXILLIPED				
Exopod: segments/ setae	4/2,2,2,3 pl	4/2,2,2,3 pl	4/2,2,2,3 pl	3/2,2,4 pl
THIRD MAXILLIPED				
Exopod: segments/ setae	4/2,2,2,3 pl	5/2,2,2,2,3 pl	5/2,2,2,2,3 pl	4/2,2,2,4 pl
PEREIOPODS				
First	Biramous bud (very small)	Biramous bud (very small)	Biramous bud (very small)	Biramous bud
Fifth	Absent	Absent	Absent	Uniramous bud

Larval biometry

• The largest newly hatched larve recorded: *L. seticaudata* (TL of 3.17 ± 0.07 mm).

• Newly hatched larvae displayed similar maxilliped endopod length. Concerning maxilliped exopod length, *L. seticaudata* larvae displayed smallest sizes for all maxilliped.

• The ratios: CT/TL, RL/TL and Tel/TL, were not significantly different for all studied species, althought the ratio RL/CL was significantly higher for newly hatched *L. seticaudata* (P<0.0001).

• *L. amboinensis* and *L. debelius* larvae displayed the highest (Mxp1Ex/TL)+(Mxp2Ex/TL)+(Mxp3Ex/TL).

Abbreviations: CT - carapace length, RL - rostrum lenght, Tel - telson lenght, TL - total length, MxpExL - maxilliped Endopod Lenght, MxpExL - maxilliped Exopod Lenght.

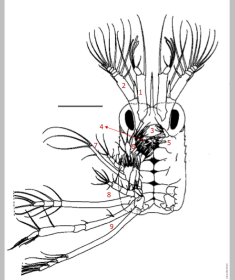

Figure 1- Newly hatched larvae of *L. amboinensis*, carapace (ventral view). Legend:1- antennula, 2- antenna, 3- labrum, 4- mandible, 5- maxillule, 6- maxilla, 7- first maxilliped, 8- second maxilliped, 9- third maxilliped. Scale bar: 0.5 mm.

Larval feeding trials

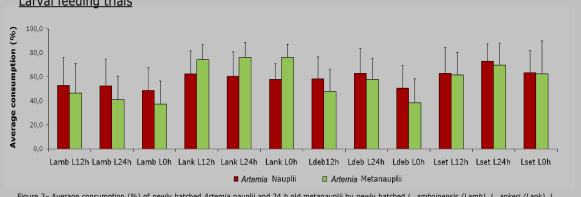

Figure 2- Average consumption (%) of newly hatched *Artemia* nauplii and 24 h old metanauplii by newly hatched *L. amboinensis (Lamb), L. ankeri (Lank), L. debelius (Ldeb)* and *L. seticaudata (Lset)* larvae under different light regimes (24, 12 and 0 hours of light) (n = 250 larvae per each species, per each type of prey and per each light regime).

Larval starvation trials

Table 2- Average survival (%) (± standard deviation) of newly hatched *L. amboinensis, L. ankeri, L. debelius* and *L. seticaudata* larvae, starved for 24, 48, 72, 96 and 120 hours and placed under different light regimes (24, 12 and 0 hours of light) (n = 5 groups of 60 larvae per each species, per each starvation period and per each light regime). Different superscript letters in the same row and different superscript numbers in the same column represent significant differences (P < 0.05). L - hours of light.

		Starvation Periods				
		24 h	48 h	72 h	96 h	120 h
L. amboinensis	L 12	80.0 ± 13.3 ^{a,1}	66.7 ± 13.3 ^{a,1}	24.4 ± 13.9 ^{b,1}	2.2 ± 0.8 ^{c,1}	0.0 ± 0.0 ^{c,1}
	L 24	66.7 ± 6.7 ^{a,1}	53.3 ± 13.3 ^{a,1}	13.3 ± 11.5 ^{b,1}	2.2 ± 0.8 ^{c,1}	0.0 ± 0.0 ^{c,1}
	L 0	66.7 ± 29.1 ^{a,1}	55.6 ± 19.2 ^{a,1}	40.0 ± 13.3 ^{a,1}	2.2 ± 0.8 ^{b,1}	0.0 ± 0.0 ^{b,1}
L. ankeri	L 12	100.0 ± 0.0 ^{a,1}	95.6 ± 3.8 ^{a,1}	73.3 ± 29.1 ^{a,23}	20.0 ± 11.5 ^{b,23}	0.0 ± 0.0 ^{c,1}
	L 24	91.1 ± 7.7 ^{a,1}	71.1 ± 23.4 ^{a,1}	48.9 ± 22.3 ^{a,123}	2.2 ± 0.8 ^{b,1}	0.0 ± 0.0 ^{b,1}
	L 0	100.0 ± 0.0 ^{a,1}	97.8 ± 3.8 ^{a,1}	82.2 ± 19.2 ^{a,23}	57.8 ± 23.6 ^{ab,2}	33.3 ± 10.6 ^{b,2}
L. debelius	L 12	82.2 ± 10.2 ^{a,1}	60.0 ± 20.6 ^{a,1}	4.4 ± 2.7 ^{b,1}	0.0 ± 0.0 ^{b,1}	0.0 ± 0.0 ^{b,1}
	L 24	73.3 ± 17.6 ^{a,1}	62.2 ± 21.5 ^{a,1}	6.7 ± 4.7 ^{b,1}	0.0 ± 0.0 ^{b,1}	0.0 ± 0.0 ^{b,1}
	L 0	68.9 ± 15.4 ^{a,1}	57.8 ± 16.9 ^{a,1}	22.2 ± 7.8 ^{b,1}	0.0 ± 0.0 ^{c,1}	0.0 ± 0.0 ^{c,1}
L. seticaudata	L 12	97.8 ± 3.8 ^{a,1}	97.8 ± 3.8 ^{a,1}	93.3 ± 6.7 ^{a,1}	42.2 ± 19.2 ^{b,2}	0.0 ± 0.0 ^{c,1}
	L 24	100.0 ± 0.0 ^{a,1}	100.0 ± 0.0 ^{a,1}	60.0 ± 26.7 ^{a,23}	6.7 ± 2.0 ^{b,1}	0.0 ± 0.0 ^{b,1}
	L 0	97.8 ± 3.8 ^{a,1}	97.8 ± 3.8 ^{a,1}	97.8 ± 3.8 ^{a,1}	66.7 ± 20.0 ^{ab,2}	22.2 ± 15.4 ^{b,2}

Swimming and feeding behaviour analysis

• Stationary and swimming *Lysmata* larvae were observed capturing and ingesting newly hatched *Artemia* nauplii and enriched metanauplii. Apparently, only preys approaching the thoracic region of the larvae, near the exopods, triggered a predatory response.

• The endopods of the maxillipeds were always the first larval appendages involved in the process of prey capture. The telson never seemed to play any role in the prey capture/manipulation process.

• The average duration of a feeding event (from prey capture to full ingestion): 52 and 145 s.

• The reports of *Lysmata* larvae thrusting forward towards dietary preys or actively chasing a larval prey were not confirmed during the present work.

Main Conclusions

• Newly hatched *Lysmata* morphology and biometry does not seem to provide reliable clues on larval prey size suitability, since this features appears to be species-specific.

• Existence of facultative primary lecithotrophy - larvae displaying this ability apparently are able to capture larger preys (e.g. *Artemia* metanauplii).

• The ability of *Lysmata* larvae to capture larger preys opens good perspectives for the use of inert diets at early stages.

• Providing enriched larval preys immediately after hatching avoids the metamorphosis delay and/or mortality.

• The manipulation of light regimes is not a viable option to increase the ingestion of larval preys.

Acknowledgements

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