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How to sample larval fish for taxonomical and ecophysiological studies in shallower temperate coastal ecosystems?

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Abstract: Recruitment predictions for fish are often difficult to make due to the intrinsic variability of species and the incomplete sampling procedures. The aim of this study was to analyse the fish larval catches (abundance, diversity, size and damage) from a standard 500 μm ichthyoplankton net and different light traps (structure, size and light intensity) in temperate coastal turbid waters. Catches from different gears were always made on the same date or season, same location and same tidal phase. Results showed that light traps captured significantly bigger larvae (almost post-flexion) than those captured with the ichthyoplankton net, nevertheless the diversity was lower. Clupeidae species were the most represented taxon; however, the traps also caught Atherinidae, Gobiidae, Sparidae, Soleidae and Labridae. The light traps were less stressful devices, allowing the capture of live and active larvae. This showed other possible uses for the light trap, e.g. larval behavioural and physiological studies. Light traps constitute a good complementary sampling option for post-flexion larvae, being aware of species selectivity associated with their use.

Résumé : *Comment échantillonner les larves de poisson pour des études taxonomiques et écophysiologiques dans des écosystèmes côtiers tempérés peu profonds ?* Les prévisions de recrutement des poissons sont souvent difficiles à réaliser en raison de la variabilité intrinsèque des espèces mais aussi en raison de lacunes dans les procédures d'échantillonnage. L'objectif de cette étude est d'analyser les captures de larves de poisson (abondance, diversité et taille) avec un filet ichtyoplancton (500 μm) et un piège de lumière dans des eaux côtières tempérées. Les échantillons ont été réalisés toujours à la même date ou à la même saison, au même endroit, et lors de la même phase de marée. Les résultats montrent que les pièges lumineux capturent les larves significativement plus grosses (presque au stade de post-flexion) que le filet ichtyoplancton, mais la diversité est plus faible. Les Clupeidae sont les espèces les plus représentées (taxons), néanmoins, les pièges ont aussi récolté des Atherinidae, Gobiidae, Sparidae, Soléidés et Labridae. Les pièges lumineux sont des dispositifs moins stressants qui permettent la capture de larves vivantes et actives. Ceci montre d'autres utilisations possibles pour les pièges à lumière, par exemple pour l'étude du comportement des larves et les études physiologiques. Des pièges lumineux constituent une bonne option d'échantillonnage complémentaire pour des larves au stade de post-flexion, étant conscient de la sélectivité des espères liée à leur utilisation.

Keywords: Larval fish sampling techniques ● Design of light trap ● Estuary ● Diversity ● Fish larval damage

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Introduction

Shallower temperate coastal ecosystems, such as estuaries and coastal lagoons, provide habitats with suitable growth conditions for many marine fish species of commercial interest, i.e. high food abundance, refuge from predators and high water temperature, and serving as important nursery grounds (Beck et al., 2001). To improve the understanding of the recruitment process based on larval fish interacting with their biotic and abiotic environment, various sampling methods, for different purposes, are commonly applied to the complex assemblages of early-life-history stages of fishes, e.g. abundance estimation, diversity studies, condition analysis in different locations, such as open sea or coastal ecosystems. To catch larval fish, horizontal, vertical or oblique tows of fine-meshed plankton net are usually towed for a relatively long period in order to collect samples of sufficient size (Smith & Richardson, 1977). However, these methods are usually more effective in sampling early larval phases (Doherty, 1982; Hickford & Schiel, 1999).

Morais et al. (2009) urge larval ichthyologists to consider the information that is being lost when only the traditional zooplankton gear and sampling strategies are used in studies of temperate estuarine ichthyoplankton. The development of sampling methods, such as light traps in coral reefs (Ruck, 1975; Doherty, 1982) and freshwater ecosystems (Nagiec, 1975) showed that it is feasible to capture in these ecosystems advanced fish larval phases, with considerable swimming ability (Leis, 2006). Nevertheless, successful studies, such as that of Strydom (2002), in a warm temperate estuary showed that it is also possible to sample with light traps in this kind of habitat.

According to Doherty (1987), the use of light traps in coral reefs can overcome the problem of subsampling post-flexion larvae and they make adequate quantification of competent larval fish more related to future, successful recruitment (Leis, 1982). The light trap assumptions are based on larval phototaxis. Light seems to be the main stimulus that governs or influences fish behaviour related to feeding and schooling (Blaxter & Parrish, 1965). In fact, the larvae of several marine fish have been described as 'photopositive', swimming persistently towards light (Blaxter & Parrish, 1965; Burke et al., 1995; Gisbert & Williot, 1997). In inshore temperate waters there are also references to the evaluation of the performance of light traps for sampling larval fish in reef and sand habitats (Hickford & Schiel, 1999).

The major problem found with net sampling was that it did not allow capture of live larvae and also it damaged individual larvae. In fact, according to Dänhardt & Temming (2007), net sampling can affect gut content and physical parameters, such as size, development of fins, notochord flexion and eyes, which are used to assess the developmental stage, swimming ability and growth of larval fish. Larval fish frequently have empty guts when collected at sea, irrespective of the time of day when they are collected. Is this the result of stress-induced defecation owing to handling in the net or preservation, or is it an indication of either individual variation in feeding activity within a patch of larvae or of a poor feeding environment (Govoni, 2005)? Moreover, there is a need to further evaluate existing and more commonly used sampling methods for post-flexion larvae from other aquatic environments that allow them to be caught in representative numbers and in a representative ecophysiological condition in the marine environment.

There are very few studies of new methodologies in temperate waters, e.g. jetski-based plankton towing in warm shallow marine habitats (Strydom, 2007) or using an underwater scooter with a plankton net for sampling in extremely rocky nearshore environments (Beldade et al., 2006). Such studies are rare in temperate turbid, shallow ecosystems, such as estuaries and coastal lagoons (Strydom, 2002), and the high suspended matter in such temperate shallow systems has somehow discouraged the use of light traps. This is because the trapping success is dependent on the turbidity of the water and the current velocity (Doherty 1987). Both are high in estuaries and coastal lagoons, such as the Gulf of Cadiz.

The aim of this study was to analyse the fish larval catches (abundance, diversity, size and damage) of a standard 500 µm ichthyoplankton net and different light trap designs (strength of light source, size and material) in temperate coastal turbid waters (Gulf of Cadiz), in order to select an adequate sampling procedure that guaranteed the representation of all fish larval phases in good physical condition.

Materials and Methods

Study site and sampling methodology

The system studied is located in the Ria Formosa coastal lagoon in the Gulf of Cadiz (South Portugal) (Fig. 1A). The Ria Formosa is a shallow (1-3 m), tidal, coastal lagoon with a high rate of water exchange with the sea (average 45-75%) over each tidal cycle (Falcão & Vale, 2003). This system is highly productive and provides ideal conditions as a fish nursery (Ribeiro et al., 2008). It is characterized by high turbidity: 15-26 $mg.l^{-1}$ (Chícharo et al., 2001).

Samples were collected with different sampling gear: different designs of light traps (structure, size and light intensity), and an ichthyoplankton net. All catches using the various gear were made controlling for the effects of date, location and tidal phase.

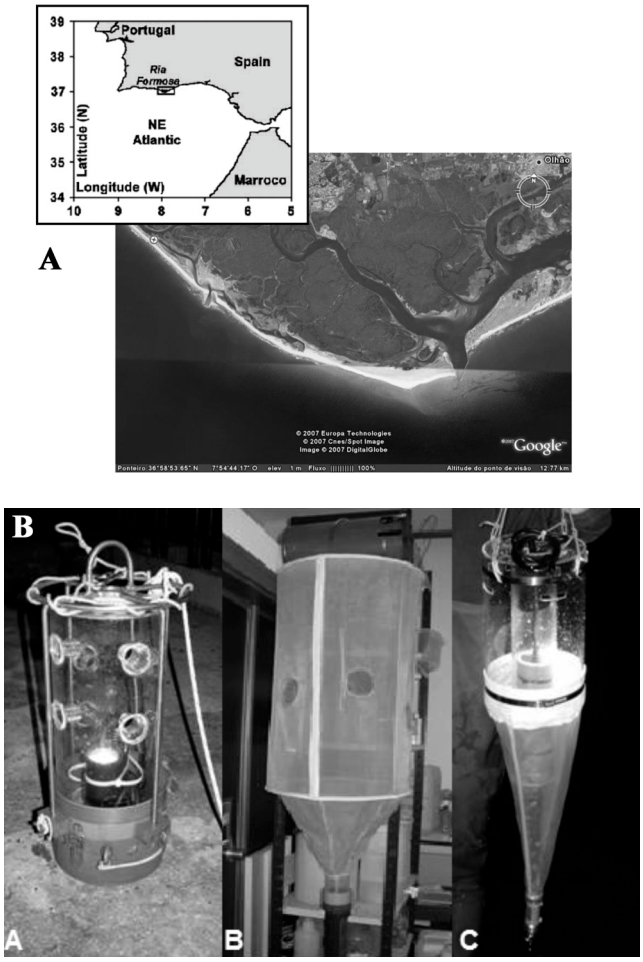

Figure 1. A - Sampling area (circle-station). B - Light traps: Photographs of trap A (Acrylic trap), trap B (Net trap), and trap C (mix trap).

Figure 1. A - Aire d'échantillonnage (Station en cercle). B - Piège de lumière: Photographies du piège acrylique (A), Filet (B) et piège mixte (C).

Between October 2005 and July 2006, sampling with light traps and the ichthyoplankton net took place in the Ria Formosa. Horizontal ichthyoplankton net tows were made at a constant speed of 2 knots at 1 m depth with a conical net (1.60 ↔ 0.37 m, 500 µm mesh-size) type WP2, equipped with a Hydro-Bios flowmeter, for 5-10 min at high or low tide (average volume of filtered water per haul: 61.2 m^3 ± 35.43).

Three light trap designs were tested (Fig. 1B). The first light trap (A-acrylic trap) consisted of an acrylic pipe 25 cm in diameter and 50 cm high, at the base of which was a 30 cm PVC bucket with four small (3 cm diameter) openings covered with 500 µm mesh. The acrylic pipe to lead to a large bucket (27 cm) leaving the larvae in a considerable water-filled space, even when it was pulled out of the sea (Fig. 1B-A). The second device to be tested was a net light trap (B) developed at the Instituto de Psicologia Aplicada (ISPA) (Fig. 1B-B) and successfully used on a rocky reef off West Iberia (Borges, 2006). It was a bigger trap (1.2 m ↔ 0.6 m), made completely of 1 mm mesh ichthyoplankton netting with no acrylic used in its construction. It had eight holes in the net material and also had a bottle-neck (Fig. 1B-B). The third trap (C-mix trap) was developed in a similar way as the first but with a rectangular hole in the acrylic pipe with a 4 cm PVC net bucket at the end, which allowed the water to pass through the mesh very quickly when the trap was pulled out of the water (Fig. 1B-C). Two different light intensities were tested in trap A: 8 W and 50 W (both white, cool, compact, fluorescent lamps).

All light traps were tested for 45 to 60 minutes. We sampled in open channels from a bridge at distinct phases of the moon cycle and at low (1-2 m depth) and high tides (3-5 m depth). The other phases of the tidal cycle (flood/ebb) were not tested because it was considered that high current velocities did not allow larvae to approach the trap, even if it was detected.

Larval fish collected by the ichthyoplankton tow or light traps were quickly sorted in a black tray and immediately frozen in liquid nitrogen (−197º C).

The absence/presence of eyes, the intestine and the overall appearance of fins (as defined by the intactness of the fins) were noted to evaluate the level of damage suffered within each of the sampling gear. The percentages of live larvae caught in each gear and the percentages of larvae in each damage class in each gear were also determined (0-no damage, 1-fins or gut or eyes structurally affected, 2-two of the anterior characteristics damaged, 3-severely affected (all previous characteristics damage))

Laboratory and data analysis

In the laboratory, larvae were identified to the lowest taxonomic level and standard length measured to the nearest 0.1 mm under a dissecting microscope equipped with an ocular micrometer. The homogeneity of variances and normality of data were tested using Levene's test and the Kolmogorov-Smirnov *k* test, respectively. Differences in catches among gear, sampling strategies and species were compared using ANOVA (Sokal & Rohlf, 1981). Shannon-Weaver diversity was determined for samples.

Results

Larval fish (n = 370) were collected in 38 samples during the night, six with a net and 32 with a light trap (Table 1).

Three light trap designs were tested and there was no significant difference for total catch (p = 0.324) and diversity (p = 0.756). In situ measurements of the 8 W lamp

light trap with a radiometer at 10 cm and 1.5 m along the trap were, on average, 618.75 (± 64.70) lux and 39 (± 64.70) lux, respectively. *In situ* measures of the 50 W light trap with a radiometer at 10 cm and 1.5 m along the trap were, on average, 3867.19 (± 404.38) lux and 243 (± 66.70) lux, respectively. There was no significant difference between light emitted at different high and low tides.

The effects of light intensity on total larvae caught and diversity were not significantly different ($p = 0.219$ and 0.771, respectively) among light traps (Table 2).

There was no significant difference between tidal and moon phases for the total number of larvae ($p = 0.362$ and 0.263, respectively) and diversity ($p = 0.692$ and 0.174, respectively), nor interactions between tide and moon ($p = 0.476$-total larvae; $p = 0.810$-diversity) (Fig. 2).

The results of the comparisons between light trap A and net catches showed that the light trap allowed larvae to be caught in higher numbers, with all live specimens and larger (standard length in mm) larvae than those collected by net. The size of the larvae caught with the light trap was significantly higher ($p < 0.001$) (Table 2). Larvae captured had an average standard length (± standard deviation) of 25.6 ± 1.2 mm, almost twice as large as those captured with the ichthyoplankton net, 14.8 ± 0.8 mm, in the same period and location (Table 1, Fig. 3). Nevertheless, species diversity (Shannon-Weaver) was lower for samples collected with the light trap, but not significantly ($p = 0.298$) (Table 2). During the sampling period, 12 taxa were collected with the net and only 10 taxa with the light trap (Table 3). The clupeiform taxa, e.g. *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758), were the most abundant. Nonetheless, the light trap also captured Atherinidae, Gobiidae, Sparidae, Soleidae and Labridae. Larvae caught with light traps were alive and completely intact while larvae caught with the net were dead and were damaged according to a scale defined in Methods. Sardines were the most affected in their morphology by the net tow. Comparisons between the light traps A and C showed that light trap A caught more live larval fish with no physical damage (Table 4).

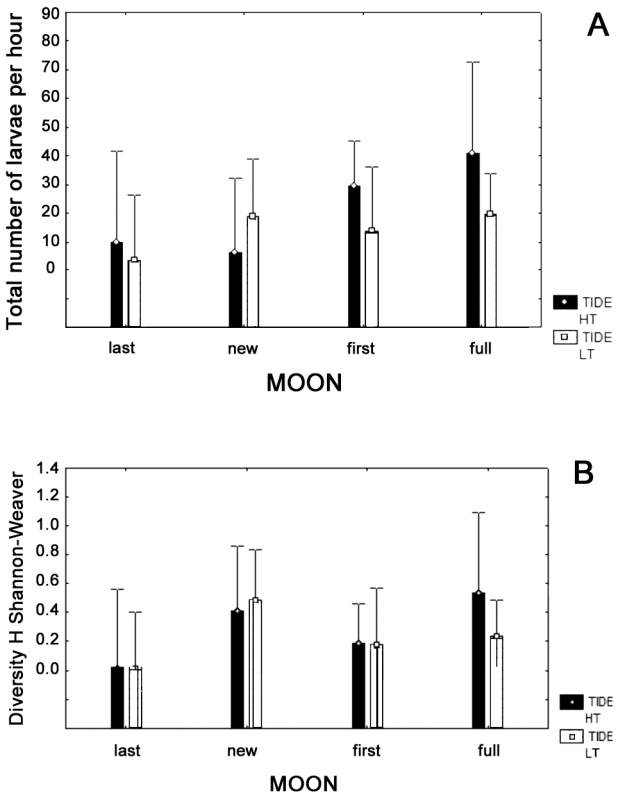

Figure 2. Comparisons between total numbers of larvae caught (A) and diversity (B) with the light trap at high and low tide and moon phases.

Figure 2. Comparaisons entre le nombre total des larves récoltées (A) et leur diversité (B) capturées avec le piège de lumière à différente phase de marée et d'endroit.

Table 1. Sampling dates and type of gear used to collected fish larvae.

Tableau 1. Dates d'échantillonnage et type de filet utilisé pour la collecte des larves de poissons.

Sampling date	Gear
November 7-17th 2005	Trap A light
January 14th 2006	Ichthyoplankton Net, Trap A
March 8th 2006	Ichthyoplankton Net, Trap A
March 14th 2006	Ichthyoplankton Net, Trap A
March 28th 2006	Ichthyoplankton Net, Trap A
March 29th 2006	Ichthyoplankton Net, Trap A
April 4th 2006	Trap A light
June 19–21st 2006	Trap A, Trap B, Trap C
July 5-17th 2006	Trap A, Trap B, Trap C

Discussion

The efficiency and development of sampling techniques for different fish larvae and young fish are key issues in recruitment studies. In other aquatic environments, such as tropical coastal areas, the use of light traps as a sampling method gave new insights into the patterns of recruitment variability in these systems and allowed direct sampling of late larvae over coral reefs (Leis, 1982; Doherty, 1987; Robertson et al., 1988; Doherty & McIlwain, 1996).

Among the light trap designs studied here, types A and C, with a light source of 8 W, were considered more adequate: for taxonomic studies (type C-4 cm PVC Net bucket) and for ecophysiological studies (type A-30 cm

Table 2. Average and Standard Deviations of standard length (SL), total number of larvae caught and diversity in the three traps tested (A, B, C), in trap A with two light powers, and in trap A and ichthyoplankton net (p-values of differences among gear).

Tableau 2. Moyenne et Déviation Standard de longueur standard (SL), nombre total de larves collectées et diversité dans les trois pièges testés (A, B, C), dans le A avec deux puissantes lumières et dans le piège A et le filet à ichtyoplancton (p : probabilité associée aux différentes entre les engins).

	SL (mm)				Total Abundance			Diversity H´			
Trap	**mean**	**std**	**N**	**p**	**mean**	**std**	**p**	**mean**	**std**	**N**	**p**
A	27.65	1.64	57	0.078	15.12	4.02	0.324	0.35	0.08	17	0.756
B	19.51	0.41	119		26.17	13.31		0.20	0.14	6	
C	18.55	0.63	50		31	8.41		0.32	0.20	9	
	SL (mm)				Total Abundance			Diversity H´			
Light trap A	**mean**	**std**	**N**	**p**	**mean**	**std**	**p**	**mean**	**std**	**N**	**p**
8 W	16.23	5.78	23	< 0.001	13.43	4.25	0.219	0.34	0.08	14	0.771
50 W	5.56	3.45	32		27	13.43		0.24	0.14	3	
	SL (mm)				Total Abundance			Diversity H´			
Gear	**mean**	**std**	**N**	**p**	**mean**	**std**	**p**	**mean**	**std**	**N**	**p**
Trap A	25.64	1.18	287	< 0.001	16.75	4.38	0.298	0.25	0.08	16	0.298
Net	14.84	0.70	52		4.17	1.01		0.40	0.14	6	

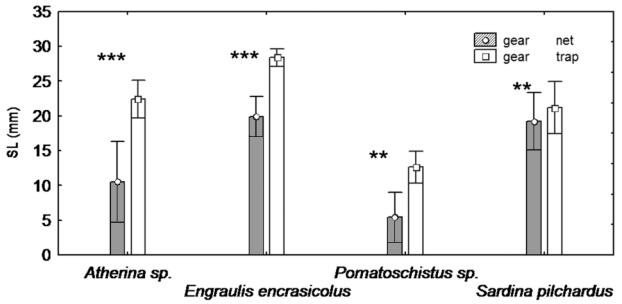

Figure 3. Standard length of the four most important larval fish species captured with the light trap and the net (** mean significant differences at $p < 0.001$, *** mean significant differences at $p < 0.005$).

Figure 3. Taille standard des quatre plus importantes espèces capturées avec un filet ichtyoplancton (500 µm) et le piège de lumière. (** différences moyennes significatives à $p < 0{,}001$, différences moyennes significatives à $p < 0{,}005$)

PVC bucket). Type C is more easily operated and Type A catches all larvae alive with no damage to them due to the wide structure and keeping the larvae in a large space in plenty of water when it is pulled out of the sea.

The traps were made smaller than usual (Doherty, 1987) to overcome problems with the low depth typical of meso-tidal temperate coastal and estuarine areas. The large size of trap B was considered disadvantageous when operating in depths of 1-2 m because of its size. Clogging in the net material lowered light transmission. The results showed no clear effects of tide and moon phase. These results were different from previous studies (Hickford & Schiel, 1999), where most common taxa were more abundant in the light trap samples during the new moon than the full moon. Our results could be explained by the high turbidity of the system studied. In fact, there was no significant difference between light emitted measured at high and low tides.

Although quantitative comparisons of early stage fishes cannot be made between plankton tows and light traps (Strydom, 2002), tentative qualitative comparisons of larval fish size, species composition and diversity can be made between these gear types. The comparison between catches from the ichthyoplankton net and the light traps showed that the light traps were more selective but they were also more efficient than the towed net in capturing bigger larvae. The underrepresented early stages in the light trap could mean these phases were not positively photo-tactic and not stron enough to swim toward it. Lower efficiency could explain the avoidance of late fish larvae to the towed net (Smith & Richardson, 1977). Nevertheless, net avoidance is lessened at night, either through reduced visual avoidance or reduced activity levels (Brander & Thompson, 1989). Therefore, by using both methods at night, any differences in size distribution, due to net avoidance alone, should be lessened. The taxonomic composition of larval fish in the samples was clearly dependent on the sampling method. The plankton net captured more taxa than the light traps, but many of these taxa were rare in the samples. Previous studies comparing light traps and plankton nets in marine waters found that light traps collected fewer families than associated plankton

Table 3. List of species, total abundance and percentages of fish species captured by light trap and net during 2005 and 2006.

Tableau 3. Liste des espèces, abondance totale et pourcentages des poissons capturés par des pièges de lumière et filet en 2005 et 2006.

TRAP—Species	**Total**	**%**
Sardina pilchardus (Walbaum, 1792)	120	41.1
Engraulis encrasicolus (Linnaeus, 1758)	72	24.7
Atherina sp.	37	12.7
Pomatoschistus sp.	50	17.1
Diplodus sp.	7	2.4
Syngnathus acus (Linnaeus 1758)	2	0.7
Tripterygion delaisi Cadenat & Blanche, 1970	1	0.3
Taxa not identified	1	0.3
Solea senegalensis Kaup, 1858	1	0.3
Blenid unidentified	1	0.3
TOTAL	**292**	**100**
NET—Species	**Total**	**%**
Pomatoschistus sp.	27	36.0
Engraulis encrasicolus	23	30.7
Sardina pilchardus	10	13.3
Atherina sp.	5	6.7
Syngnathus acus	2	2.7
Soleid unidentified	2	2.7
Diplodus vulgaris (Geoffroy St Hilaire, 1817)	2	2.7
Diplodus sp.	2	2.7
Taxa not identified	2	2.7
Pomatoschistus pictus (Malm, 1865)	1	1.3
Parablennius gattorugine (Linnaeus, 1758)	1	1.3
Belone belone (Linnaeus, 1761)	1	1.3
TOTAL	**75**	**100**

tows (Choat et al., 1993; Brogan, 1994; Hickford & Schiel, 1999). Nevertheless, Strydom (2002) in a study in a warm estuary in South Africa found most species caught in the plankton net studies were also represented in light trap catches. Our results, however, showed species did vary greatly between plankton net and light trap catches. The lower diversities obtained from samples collected by light traps may result from different behaviour in response to light by different larval fish species, in different development phases (Burke et al., 1995; Gisbert & Williot, 1997; Giannoulaki et al., 1999). The light trap captured advanced stages of post-flexion larval fish of different species, mainly *Sardina pilchardus, Engraulis encrasicolus, Atherina* spp.*, Pomatoschistus* spp. Clupeids were the most abundant species, which is the common pattern in Portuguese coastal areas (Ré et al., 1990; Ré, 1996; Santos et al., 2007). There are also earlier references of clupeid larvae caught by light traps in tropical areas (Baker, 1972; Davies, 1954) This could also have been due to the preferred light intensity of this species-25-100 lux (Giannoulaki et al., 1999), which is close to the average radiation measured around light traps (up to 1 m) in the present study. An important aspect when using light traps is the knowledge of photaxis behaviour of species and this is not always described in the literature.

Net sampling can affect gut content and physical parameters such as the size, development of fins, notochord flexion and eyes, which are used to assess the developmental stage, swimming ability and growth of fish larvae (Dänhardt & Temming, 2007).

All of the above-mentioned assessments require intact larvae, which are not always guaranteed using commonly net sampling methods. The use of light traps as sampling devices minimizes the physiological and morphological stress, e.g. gut evacuation, missing eyes, and body shrinkage. It may bring new insights into larval ecology studies, especially because it allows catching live larvae, enabling behavioural studies, e.g. critical swimming speed, sustainable swimming, and physiological studies, e.g. evacuation, excretion, ingestion or respiration rate, and studies with species in which the life cycle is difficult to complete successfully in culture conditions, e.g. clupeids and engraulids. Another advantage of light traps over plankton nets is the ease with which light trap samples can be simultaneously replicated. On most sampling occasions replicated light trap samples were less variable than the associated plankton tows. This reduced variance will make the light traps, when used in a structured design, more sensitive to detecting differences among treatment means (Hickford & Schiel, 1999)

Conclusions

It can be concluded that light traps underestimated early larval stages and taxonomic diversity while net tows underestimated post-flexion stages and damaged more fish larvae in catches. A solution may be achieved by assembling information inferred from larvae collected with ichthyoplankton nets and with light traps specially designed to operate in coastal areas to capture photopositive larval fish. In fact, the light traps developed in this study will contribute to a better comprehension of ecological processes structuring fish populations that use shallow and turbid temperate systems as nursery areas. This is because it allows the capture of post-larvae in good physical condition necessary to estimate potential survival through ecophysiological studies and future recruitment. In fact, in order to understand recruitment variability of fish in shallow temperate ecosystems, adequate sampling of all fish larval phases and their ecophysiological condition is essential.

Table 4. Percentage of live larvae caught in each gear and percentage of larvae in each damage class in each gear (0-no damage, 1-fins or gut or eyes structurally affected, 2-two of the anterior fins damaged, 3-severely affected (all previous damage)).

Tableau 4. Pourcentage de larves vivantes récoltées dans chaque filet et pourcentage de larves abimées dans chaque classe (0 : aucun dommage ; 1 : nageoires ou estomac ou yeux structurellement affectés ; 2 : deux des nageoires antérieures endommagées ; 3 : affecté sévèrement (tous les dommages précédents).

TRAP A – Species	**Total**	**% alive**	**Damage**			
			0	1	2	3
Pomatoschistus sp.	16	(6/16) 37.5%	100%	0%	0%	0%
Engraulis encrasicolus	42	(34/42) 81%	100%	0%	0%	0%
Sardina pilchardus	23	100%	100%	0%	0%	0%
Atherina sp.	7	100%	100%	0%	0%	0%
Diplodus sp.	7	100%		0%	0%	0%
TRAP C – Species	**Total**	**% alive**	**Damage**			
			0	1	2	3
Pomatoschistus sp.	28	100%	28%	0%	0%	0%
Engraulis encrasicolus	15	100%	15%	0%	0%	0%
Sardina pilchardus	30	100%	30%	0%	0%	0%
Atherina sp.	30	100%	30%	0%	0%	0%
NET – Species	**Total**	**% alive**	**Damage**			
			0	1	2	3
Pomatoschistus sp.	27	0%	0%	70% (19/27)	30% (8/27)	0
Engraulis encrasicolus	23	0%	0%	78% (18/23)	13%(3/23)	1% (2/23)
Sardina pilchardus	10	0%	0%	50% (5/10)	20% (2/10)	30% (3/10)
Atherina sp.	5	0%	0%	80% (4/5)	20% (1/5)	0

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References

Baker A.N. 1972. Reproduction, early life history, and age-growth relationships of the New Zealand pilchard, *Sardinops neopilchardus* (Steindachner). *Fisheries Research Bulletin*, **5**: 64 pp.

Beck M., Heck K., Able K., Childers D., Egglestone D., Gillanders B., Halpern B., Hays C., Hoshino K., Minello T., Orth R., Sheridan P. & Weinstein M. 2001. The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience,* **51**: 633-641.

Beldade R., Borges R. & Gonçalves J.E. 2006. Depth distribution of nearshore temperate fish larval assemblages near rocky substrates. *Journal Plankton Research,* **28**: 1003-1013.

Blaxter J.H.S. & Parrish B.B. 1965. The importance of light in shoaling, avoidance of nets and vertical migration by herring. *Journal Conseil Permanent International pour l'Exploration de la Mer*, **30**: 40-57.

Borges R. 2006. Composition, temporal and spatial patterns of very nearshore larval fish assemblages at the Arrábida Marine Park. Ph.D. thesis, University of Algarve, 343 pp.

Brander K. & Thompson A.B. 1989. Diel differences in avoidance of three vertical profile sampling gears by herring larvae. *Journal of Plankton Research,* **11**: 775-784.

Brogan M.W. 1994. Two methods of sampling larval fish over reefs: a comparison from the Gulf of California. *Marine Biology*, **118**: 33-44.

Burke J., Seikai T. & Tanaka M. 1995. Influence of light and salinity on behaviour of larval Japanese flounder (*Paralichthys olivaceus*) and implications for inshore migration. *Netherlands Journal of Sea Research*, **34**: 59-69.

Chícharo L., Chícharo M.A., Gaspar M.B., Labarta U., Marin L., Pellizato M., Reiriz da M.J., Ros M. & Vafidis D. 2001. Impact of bivalve fisheries in the Ria Formosa lagoon (Portugal), Venice lagoon (Italy) and Aegean sea (Greece): an integrated overview. *ICES CM 2001/J*: 08, 30 pp.

Choat J.H., Doherty P.J., Kerrigan B.A. & Leis L.M. 1993. A comparison of towed nets, purse seine, and light-aggregation devices for sampling larvae and pelagic juveniles of coral reef fishes. *Fisheries Bulletin*, **91**: 195-209.

Dänhardt A. & Temming A. 2007. Effects of haul duration on the physical condition of sprat larvae. *Aquatic Biology*, **1**: 135-139.

Davies D.H., 1954. The South African pilchard (*Sardinops ocellata*). Development, occurrence and distribution of eggs and larvae, 1950-51. *Investigational Report of the Division of Fisheries. Union of South Africa*, **15**: 23-58

Doherty P.J. 1982. Some effects of density on the juveniles of two species of tropical territorial damselfishes. *Journal of Experimental Marine Biology and Ecology*, **65**: 249-261.

Doherty, P.J. 1987. Light-traps: selective but useful devices for quantifying the distributions and abundances of larval fishes. *Bulletin of Marine Science*, **41**: 423-431.

Doherty, P.J. & McIlwain J.L. 1996. Monitoring larval fluxes through the surf zones of Australian coral reefs. *Marine and Freshwater Research*, **47**: 383-39.

Falcão M. & Vale C. 2003. Nutrient dynamics in a coastal lagoon (Ria Formosa, Portugal): the importance of lagoon-sea water exchanges on the biologic productivity. *Ciencias Marinas*, **29**: 425-433.

Giannoulaki M.A., Machias M. & Tsimenides N. 1999. Ambient luminance and vertical migration of the sardine *Sardina pilchardus. Marine Ecology Progress Series*, **178**: 29-38.

Gisbert, E. & Williot P. 1997. Larval behaviour and effect of the timing of initial feeding on growth and survival of Siberian sturgeon (*Acipenser baeri*) larvae under small scale hatchery production. *Aquaculture*, **156**: 63-76.

Govoni J.J. 2005. Fisheries oceanography and the ecology of early life histories of fishes. *Scientia Marina*, **69**: 125-137.

Hickford M.J.H. & Schiel D.R. 1999. Evaluation of the performance of light traps for sampling larval fish in inshore temperate waters. *Marine Ecology Progress Series*, **186**: 293-302.

Leis J.M. **1982.** Nearshore distributional gradients of larval fish (15 taxa) and planktonic crustaceans (6 taxa) in Hawaii. *Marine Biology*, **72**: 89-97.

Leis J.M. 2006. Are larvae of demersal fishes plankton or nekton. *Advances in Marine Biology,* **51**: 59-141.

Morais P., Faria A., Chicharo M.A. & Chicharo L. 2009. The unexpected occurrence of late *Sardina pilchardus* (Walbaum, 1792) (Osteichthyes: Clupeidae) larvae in a temperate estuary *Cahiers de Biologie Marine,* **50**: 79-89.

Nagięć M. 1975. Description of a ring net and a trap net for the sampling of pike-perch fry. *EIFAC Technical Papers*, **23** (Suppl.1,vol. 2): 565-70.

Ré P. 1996. Anchovy spawning in the Mira Estuary (southwestern Portugal). *Scientia Marina*, **60** (Supl. 2): 141-153.

Ré P., Silva R.C., Cunha E., Farinha A., Meneses I. & Moita T. 1990. Sardine spawning off Portugal. *Boletim do Instituto Nacional de Investigacão das Pescas, Portugal*, **15**: 31-44.

Ribeiro J., Monteiro C.C., Monteiro P., Bentes L., Coelho R., Gonçalves J.M.S., Lino P.G. & Erzini K. 2008. Long-term changes in fish communities of the Ria Formosa coastal lagoon (southern Portugal) based on two studies made 20 years apart. *Estuarine Coastal Shelf Science*, **76**: 57-68.

Robertson D.R., Green D. & Victor B.C. 1988. Temporal coupling of production and recruitment of larvae of a Caribbean reef fish. *Ecology*, **69**: 370-381.

Ruck J.G. 1975. A Light-Trap for Collecting Marine Fish Larvae and Invertebrates. *Tuatara*, **21**: 98-104.

Santos A.M.P., Chícharo M.A., Dos Santos A.T., Moita A., Oliveira P.B., Peliz Á. & Ré P. 2007. Physical-biological interactions in the life history of small pelagic fish in the Western Iberia Upwelling Ecosystem. *Progress in Oceanography*, **74**: 192-209.

Strydom N.A. 2002. Dynamics of early stage fishes associated with selected warm temperate estuaries in South Africa. *PhD Thesis Rhodes University*. 166 pp.

Strydom N.A. 2007. Jetski-based plankton towing as a new method of sampling larval fishes in shallow marine habitats. *Environmental Biology of Fishes*, **78**: 299-306.

Smith P. & Richardson S. 1977. Standard techniques for pelagic fish egg and larva surveys. *FAO Fisheries Technical Papers*, **175**: 100 pp.

Sokal R.R. & Rohlf F.J. 1981. *Biometry. The principles and practice of statistics biological research.* 2nd Edition. W.H. Freeman: New York. 148 pp.