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Abstract

The pilotfish (Naucrates ductor) is an epipelagic oceanic species, whose 0-group specimens tend to aggregate below flotsam, constituting a principal by-catch of the western Mediterranean FAD fishery. During a one-year survey sampling, monthly experimental hauls using surrounding nets were conducted at sites in south Peloponnesian waters, where FADs were moored; during the summer-early autumn months bongo tows were also carried out. 0-group pilotfish appeared beneath FADs from mid-summer till early winter and the total length of the collected specimens ranged between 120 and 330 mm. Pilotfish larvae, measuring 2.0-3.9 mm, were found at FAD sites in mid-summer and in early autumn. The age of juvenile specimens, determined by counting daily increments on their sagittae, ranged between 50-141 increments (days) for males, and 51-131 increments (days) for females. The predicted asymptotic length was found to be 412.3 mm TL for males and 435.2 mm TL for females. The onset of sexual maturity appeared to occur when specimens were a few months old. Stomach content analysis suggested that the diet of 0-group pilotfish associated to FADs in Greek waters depended mainly on decapod larvae, hyperiid amphipods, and alciopid polychaetes.

Keywords: Daily rings; Maturity; Diet; 0-group pilotfish; Greek waters.

Introduction

Thigmotropism in fish (the attraction to a solid object) is a well-documented phenomenon. Many factors acting on the individuals and several mechanisms have been suggested to explain the association of fishes with floating objects. Four of the more accepted mechanisms are: shelter from predators, food supply, schooling companions and substrate for species undergoing a change from a pelagic to other modes of existence (GOODING & MAGNUSON, 1967; HUNTER & MITCHELL, 1967; WICKHAM *et al.*, 1973; WICKHAM & RUSSEL, 1974; MATSUMOTO *et al.*, 1981; ROUNTREE, 1989; FONTENEAU, 1993). Association of fishes with flotsam is a phenomenon that has been used widely to enhance local fisheries (SHOMURA & MATSUMOTO, 1982; DELMENDO, 1990).

The use of floating fish aggregating devices (FADs) to attract fishes has been a diffused activity in the Mediterranean since antiquity (ANNA *et al.*, 1999). The pilotfish *(Naucrates ductor)* is considered the most characteristic species of the fish assemblage associated with floating objects in the Mediterranean, due to its year round presence near flotsam (MASSUTI & RENONES, 1994). Although juvenile specimens are those often found below floating objects, adults are commonly associated with large-sized living animals, such as sharks, rays and turtles. The pilotfish is an epipelagic oceanic species that is almost cosmopolitan in tropical and subtropical seas. Along with the amberjack *(Seriola dumerili)* they constitute the principal by-catch species of the western Mediterranean FAD fishery targeting dolphinfish *(Coryphaena hippurus)* (RENONES *et al.*, 1999).

The biology of this species is poorly known and very few studies have been carried out in the Mediterranean. In particular, RELINI *et al.* (1994) reported on the length frequency distribution of specimens associated with an offshore buoy in the Ligurian Sea, RENONES *et al.*, (1998) carried out a preliminary study of its biology and RENONES *et al.*, (1999) studied the population structure and the age and growth of specimens collected in FAD fisheries of Mallorca Island. Information on its feeding habits in western Mediterranean regions is provided by RELINI *et al.* (1994), VASKE (1995), RENONES *et al.* (1998), PIPITONE *et al.* (2000) and DEUDERO & MORALES-NIN (2001). The present study aims to provide information on the size structure, age, growth, maturity, and feeding habits of the juvenile pilotfish collected beneath FADs in eastern Mediterranean (Greek) waters.

Materials and Methods

Sampling took place off the Messinian coast in the southwest Peloponese (3642,3-3647,1°N – 2147,4-2159,7°E). In late July 2000, a total of 24 FADs constructed either by using palm branches tied together, or by using a block of expanded polystyrene, 10mm thick, having an average length of 150 mm and a width of 100 mm, were set at a distance of about 500 m from each other in sites with depths of about 60-200 m. FADs' positions were often visited during the study period, that lasted from July 2000 till June 2001 and in that period once a loss of a FAD was reported, a new construction was deployed.

Fishing was conducted in the FAD areas on a monthly basis, using a surrounding net having an altitude of 25 m and a mesh size of 40 mm in the mid-area, which is typical of the FAD fishery in Sicilian waters. At each haul, the total catches, as well as species' composition by weight and number, were determined. For laboratory analysis, the whole sample of the pilotfish was stored, if less than 50 specimens were collected, while in the cases when more than 50 specimens were fished, a representative sub-sample was taken. Samples that would be used for age readings were preserved in a deep freeze, while those for dietary analysis were stored in plastic jars containing 10% formaldehyde solution.

During the laboratory analysis, for each pilotfish the total length (TL, mm), the standard length (SL, mm), the total somatic weight (W, g), the eviscerated weight (We, g), the gonad weight (Wg, g), the sex and the sexual maturity stage (Holden and Raitt scale: HOLDEN & RAITT (1975)) were recorded. The otoliths were removed from the head and preserved following the method of PANELLA (1980). Sagittae were used owing to their relatively larger size in comparison with the lapillus and asteriscus. Each pair of sagittae was stored in vials with distilled water for further analysis. For age readings, the sagittae were embedded in heat hardening implex resin, in order to obtain a frontal section. Sequential grinding was performed and final polishing was accomplished using a 0.3 alumina paste. Age readings were made under a light microscope, coupled to a high-resolution video camera and monitor system, following standard procedures (CAMPANA & NEILSON, 1985; MORALES-NIN, 1992). The sagittae showed the alternative dark-light ring patterns and the increments were laid down around a small oblong core. Growth increments were counted from the core to the tip of the rostrum. Incremental counts were made by beginning at the first clearly defined mark that encircled the primordium which defines the outer edge of the nucleus. RENONES *et al.* (1999) provided evidence on the daily nature of growth increments on pilotfish sagittae, and hence growth increments were considered as daily rings. Two independent readings were performed, and the results were accepted only if the readings were coincident or the difference in number of increments between the readings was <5%. Daily ring measurements were made at 400x magnification. The relation between fish total length and sagittal daily rings was determined with a predictive linear regression of length on number of rings (RICKER, 1975; FRANCIS, 1990). For a more realistic representation of growth, age-length data were also modelled by applying the von Bertalanffy growth equation using FISHPARM program (PRAGER *et. al.*, 1987).

The study of the feeding habits of the species was based on the analysis of the stomach contents of specimens. All prey items were identified to the lowest possible taxonomic level, counted and weighed (wet weight at 0.001 gr.). The count of fragmented preys was based on the number of eyes, mouth parts, tails or other anatomical parts referable to single specimens. The importance of each prey item in the diet of each species was assessed by means of the indices Cn, Cw, F (HYSLOP, 1980) and IRI (PINKAS *et al.*, 1971) as follows:

- percent numerical abundance (Cn): $Cn = (n_i / \Sigma n) \times 100$, where ni=abundance of prey i, Σn=total abundance of preys.

- percent weight (Cw): $Cw = (w_i / \Sigma w) \times 100$, where w_i=weight of prey i, Σw=total weight of preys.

- percent frequency of occurrence (F): $F = (S_i / Sf) \times 100$, where S_i=number of stomach containing prey i, Sf=number of stomach containing food.

- index of relative importance (IRI): $IRI = (Cn+Cw) \times F$, where Cn= percent numerical abundance, Cw= percent weight and F=percent frequency of occurrence.

In parallel with the fishing survey, an ichthyoplankton one was conducted in June, July and early September 2001. Plankton hauls were made using a Bongo net (60 cm) with mesh sizes of 300 and 500 μm. Hauls were taken horizontally varying from 5 to 30m depth, at a ship's speed of 2.5 knots and lasted for about 10 min. The volume of filtered water was estimated using a flowmeter. All samples were collected during daylight (09:00 to 14:00 h). After the end of each haul, samples were labeled, preserved in 4% neutralized formalin and stored in plastic vials. In the laboratory, all fish eggs and larvae were removed from each sample, identified to the lowest possible taxonomic level and enumerated. Data were standardised to number of larvae per 100 m^3 water. Recording of maximum depth and temperature had been obtained using a diving watch adapted to the Bongo net.

Results

Ichthyoplankton data

Analysis of the ichthyoplankton samples collected at the FAD sites revealed a total of 22 taxa of fish eggs and larvae, representing 16 families. Pilotfish larvae were present in samples from July and September, their abundance peaking in July, when it reached 21.23% of the total larval catch. The average surface temperature (±SD) during the surveys was recorded at 23.67 °C ± 1.25 in July and at 25.20 °C ± 0.37 in September. The length range of the collected larvae was comprised between 2 and 3.9 mm TL (Table 1).

Table 1
Abundance of the larvae of the various fish species, as well as of those of pilotfish, expressed as number of larvae per 100 m^3 water surface, and length range (mm TL) of the pilot fish collected beneath FADs.

Month	Nall	N*N.ductor*	Length range
June	76.5	-	-
July	13.0	2.76	2.00-2.90
September	91.9	0.75	2.00-3.70

Length frequency distribution

In order to make the present results comparable with those from other studies where the fork length (FL) of specimens was used, the equation converting TL (total length) to FL (fork length) is provided: TL=2.217+1.118xFL, r=0.994.

The species was present beneath FADs from July to January. In all, 357 specimens were caught, whose size range was between 120 and 330 mm TL (Fig. 1). The majority (>70%) of the collected pilotfish were specimens of 220-260 mm TL. Very few specimens smaller than 200 mm TL were collected, due to the selectivity of the net used during the study. Most of the collected small specimens were males, while specimens of 310-330 mm TL were females. The application of a one-way ANOVA did not suggest the existence of any significant difference between the mean lengths of the collected male and female fish (P>0.05).

Age and growth

Age could not be determined in 13.4% of the 201 sagittae pairs, that were extracted from fish heads, either because they could not be clearly read due to their damage during the preparation, which was particularly true for the sagittae of the smallest fish (<189 mm TL) or because the error in reading precision was greater than 5%. Thus, counts of growth increments were obtained from 174 specimens, 84 males and 90 females. In Figure 2 a micrograph of the frontal section of a pilotfish sagitta is provided. The age range determined from sagittal interpretation was 50-141 increments for males having a respective total length range of 199-305 mm (TL), and 51-131 increments for females, whose total lengths ranged between 189 and 290 mm TL. Hence, our results suggested that fish appearing beneath FADs were younger than five months old. The parameters of the linear relationships between TL and the number of increments in the sagittae appear in Table 2 and the respective plots appear in Figure 3. These plots show a marked increase in the scatter of points with age of fish. Differences in growth rate by

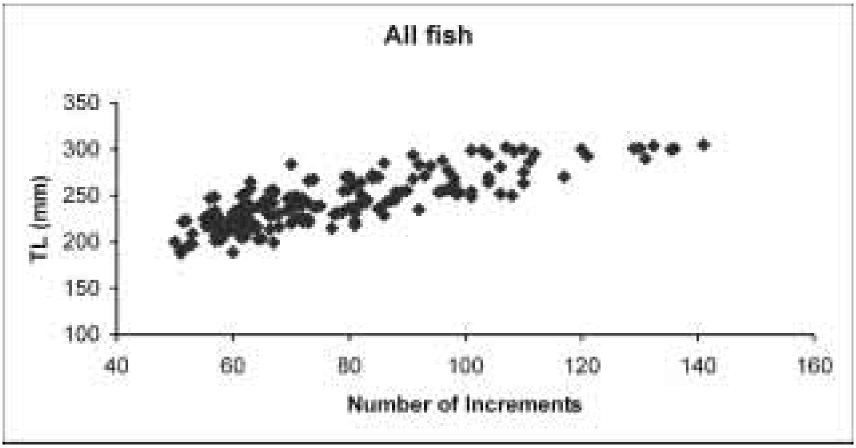

Fig. 1: Length frequency distribution of male and female pilotfish collected beneath FADs in the south Peloponese.

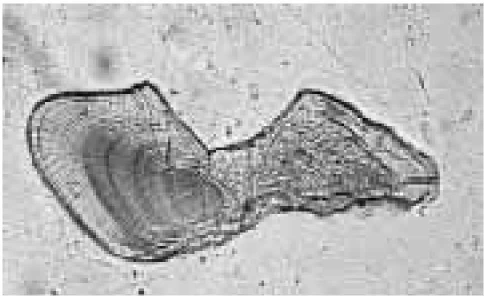

Fig. 2: Light micrograph of the frontal section of a pilotfish sagitta.

sex were not significant as revealed by the application of ANCOVA to the TL-number of increment data of males and females (P>0.05).

The parameters of the von Bertalanffy growth equation and the growth performance index appear in Table 3. The predicted asymptotic length for the species in Greek waters was found to be 435.2 mm TL for females and 412.3 mm TL for males.

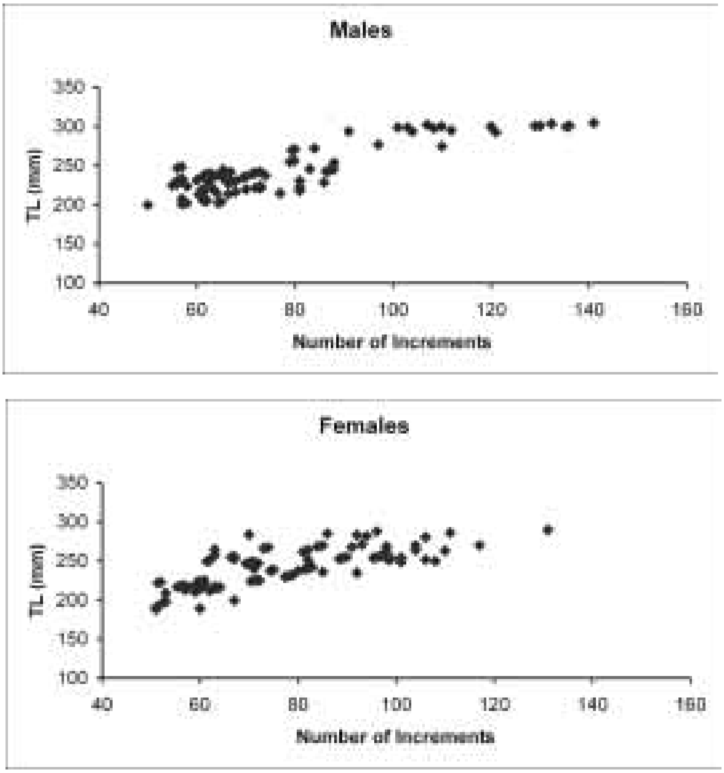

Fig. 3: Plot of the number of increments in sagittae versus total length of pilotfish from Greek waters.

Table 2
Summary of results for linear regressions of growth increments in sagittae on total lengths of pilotfish. (r=coefficient of correlation, n=number of fish).

	Slope	(SE)	Intercept	(SE)	r	n
All fish	1.14	(0.06)	154.69	(16.17)	0.82	174
Males	1.21	(0.07)	149.48	(15.40)	0.87	84
Females	1.03	(0.09)	162.66	(16.82)	0.74	90

Table 3
Von Bertalanffy growth curve parameters for pilotfish from Greek (G) waters. Standard errors of estimates are in brackets. Φ: growth performance index (Munro & Pauly, 1983).

	L_ (mm)	K (annual)	to (years)	Φ
Female	435.2	2.15	-0.164	5.609
	(44.8)	(0.97)	(0.101)	
Male	412.3	2.57	-0.127	5.640
	(31.6)	(1.09)	(0.074)	
All fish	422.0	2.25	-0.161	5.603
	(22.2)	(1.03)	(0.089)	

Isometric growth was found in the total length (TL mm) – total weight (TW g) relationship for females and negative allometric growth for males and sexes combined ($P<0.05$).

Males:

$W= 1.31x10^{-3}xTL^{2.13}$, $n= 93$, $r=0.84$

Females:

$W= 8.43x10^{-6}xTL^{3.05}$, $n= 110$, $r=0.96$

All fish:

$W= 1.49x10^{-4}xTL^{2.53}$, $n= 203$, $r=0.90$

Maturity stages and sex ratio

During the sampling period the gonads of the collected specimens were at stages I-IV of the Holden and Raitt scale (Figure 4). In general, the vast majority of the gonads were immature (stage II) while few specimens, the larger ones (TL>250 mm), were at an early maturing stage (stage III), or they had already reached their first sexual maturity (stage IV: TL>300 mm). It should be noted, however, that the latter size group exhibited few representatives (seven specimens) that were collected between July and November,

Table 4
Stomach content analysis of pilotfish caught in south Peloponnesian waters. Cn: percent abundance, Cw: percent weight, F: frequency of occurrence, IRI: index of relative importance.

Prey items	Cn	Cw	F	IRI
Annelida				
Polychaeta un.		37,73	27,27	1029,11
Alciopidae	30,49	3,37	23,64	800,44
Arthropoda				
Crustacea un.		0,08	52,73	4,33
Decapoda un.		37,65	50,91	1916,55
Decapoda larvae	20,63	2,86	47,27	1110,15
Isopoda		3,14	0,07	10,91 35,01
Natantia un.		0,12	20,00	2,48
Pasiphaeidae	0,90	0,16	7,27	7,67
Sergestidae	0,45	0,10	1,82	1,01
Stomatopoda larvae	4,93	1,22	14,55	89,54
Amphipoda un.	12,58	56,36	708,94	
Hyperiidae	14,80	2,72	34,55	605,23
Euphausiacea	0,90	0,35	3,64	4,53
Mysidacea	2,24	0,02	5,45	12,33
Mollusca				
Gastropoda				
Heteropoda	3,14	0,12	9,09	29,65
Vertebrata				
Osteichthyes eggs	13,90	0,06	29,09	406,03
Osteichthyes larvae	1,35	0,68	5,45	11,05
Tunicata				
Appendicularia	3,14	0,10	12,73	41,29

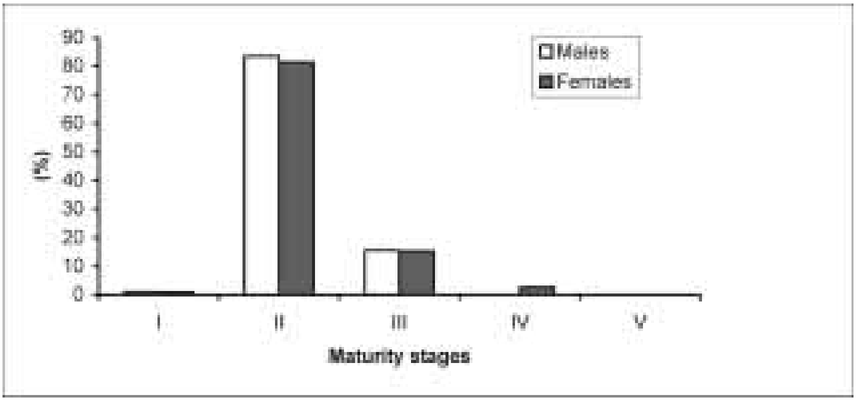

Fig. 4: Gonadal maturity of pilotfish collected during the survey samplings in Greek waters.

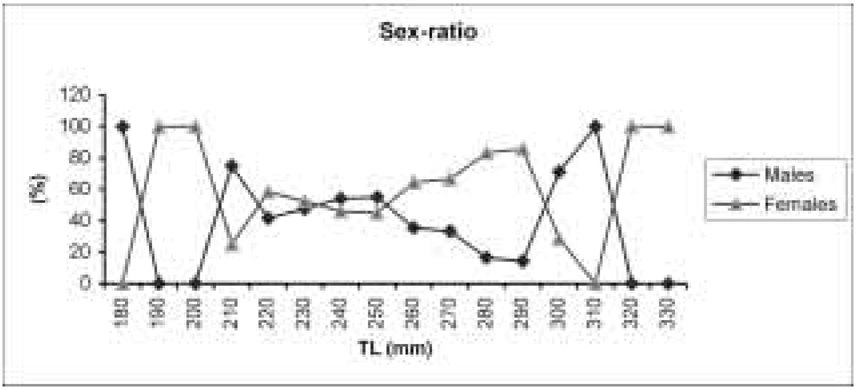

Fig. 5: Sex-ratio of pilotfish in relation to size of specimens.

underlining the preliminary character of these observations.

From the 221 specimens whose gonads were studied, 118 were females and 103 were males. The sex-ratio did not differ significantly from the 1:1 theoretical value ($x^2=0.449$, $P>0.5$). In relation to fish size, in the length interval from 220 to 250 mm, coinciding with the size range of the bulk of the collected stock the sex-ratio did not differ from 1:1 ($P>0.05$) (Figure 5). In smaller and larger sizes significant deviations appeared ($P<0.001$), which could result from the few specimens that were comprised in the respective size groups.

Feeding habits

From the stomachs of the 221 pilotfish that were analysed, 25 (11.3%) were found to be empty. The preys that were found belonged to five major preys categories i.e. Arthropoda (crustacea), Annelida (polychaeta), Mollusca (gastropoda), Vertebrata (osteichthyes) and Tunicata (appendicularia). Crustaceans, and particularly decapod larvae and hyperiid amphipods, and then Alciopidae polychaetes were the most important prey items as revealed by the Index of Relative Importance (IRI) (Table 4).

Discussion

FADs provide a useful tool for studying species and life phases that are difficult to sample using conventional methods (HUNTER & MITCHELL, 1967; KINGSFORD & CHOAT, 1985; KINGSFORD, 1992). This seems to be also the case for the pilotfish, since the present is the first report of the species' larvae, collected in Greek waters.

Pilotfish juveniles resided in schools beneath FADs in south Peloponnesian waters for a rather long period of time (July-January). According to RADAKOV (1973) the schooling behavior lessens the possibilities for predators gaining access to the shoal, although it increases them for predators, which hunt in shoals themselves. The length range of pilotfish caught beneath FADs in the study area was 120 to 330 mm TL (2 to 5 months old), although due to the selectivity of the used net the catch mainly comprised specimens with total lengths from 200 to 330 mm TL. RENONES *et al.* (1999) mentioned that specimens of similar size were collected in western Mediterranean waters in the framework of dolphinfish fishery using FADs and advocated that the species shows a size-dependent behaviour; the shoaling behaviour occurs when individual fish have significantly more foraging and anti-predator advantages while being together with conspecifics. Those advantages, however, appear to decrease with growth in length (PITCHER, 1995). RIERA *et al.* (1999) stated that the largest specimens observed under FADs off the Balearic Islands never exceeded 370 mm total length. SMITH-VANIZ (1986) revealed that in Atlantic waters the species live beneath floating objects, while adults live with sharks, big rays and other marine animals, reaching a maximum length of 700 mm. On the other hand, COUSTEAU & DUMAS (1963) observed and photographed a young pilotfish of 60-70 mm with a *Carcharinus longimanus* in the south-western Atlantic. According to RIERA *et al.* (1999) the species joins sharks and other big marine animals as soon as they can, depending on their presence or availability.

The majority of pilotfish collected at FAD sites in Greek waters were two to four months old, and the largest specimens were five months. RENONES *et al.* (1998) noted that Mallorca FAD fishery exploited pilotfish ranging from two to six months old. Our data showed a fast growth for the species during the first months of its life and the predicted asymptotic length was found to be 422 mm TL, which is larger than the value found by RENONES *et al.* (1999) for the species in western Mediterranean waters (289 mm FL). It should be noted, however, that values of the growth parameters obtained in the framework of the present study, as well as by RENONES *et al.* (1999), might be biased since they were calculated using juvenile specimens. The only existing information regarding the species' maximum longevity was provided under culture conditions, during which the pilotfish reached three years in age (RENONES *et al.*, 1999).

The gonads of the majority of the collected specimens were immature; few specimens were at an early maturing stage, or they were already mature. Thus, our data suggested that the onset of sexual maturity takes place early in the species life, since specimens larger than 300 mm had mature gonads. The latter is in accordance with the findings of RENONES *et al.* (1998) who have found mature females among specimens larger than 290 mm FL. Due to the seasonality of the catches and the limited number of larger/mature specimens, our data do not allow the extraction of reliable conclusions regarding the duration of the spawning period of the species. It seems, however, that it should be rather prolonged, extending at least from early summer, taking into account the high presentation of pilotfish larvae in the ichthyoplankton in July, till late autumn, when the last large and mature specimens were collected. Our indications on a rather prolonged spawning period of pilotfish, are in accordance with the findings of Renones *et al.* (1998), who suggested that a long spawning period might be one of the species' adaptations favouring its survival in a very variable environment.

Juvenile pilotfish collected during this study were planktivorous, the most important prey items in terms of abundance and weight in the stomachs being decapod larvae, hyperiid amphipods, and pelagic polychates. Similar findings were reported also for specimens associated with drifting objects in western Mediterranean (RELINI *et al.*, 1994; RENONES *et al.*, 1998; PIPITONE *et al.*, 2000; DEUDERO & MORALES-NIN, 2001). The absence of any fouling organisms living on the FADs (i.e. lepadi cirripeds) from the stomachs of specimens collected in the study area suggested that FADs do not seem to have any direct, at least, role in the feeding of the species. The latter is also mentioned by RENONES *et al.* (1998), who commented that pilotfish juveniles seem to forage in the epipelagic layer, taking advantage of the diel vertical migration of various zooplanktonic species. On the other hand, as fish grow, a change in their nutritional requirements appears to take place, since VASKE (1995) found a great percentage of teleostean rest in the stomach contents of large pilotfish living under marine animals. SMITH-VANIZ (1986) supported that the association of the species with living hosts may be related to trophic motives.

Data derived through this study confirm previous findings from western Mediterranean and Atlantic waters regarding the species' association to flotsam during the first months of its life. In particular, our results suggested that pilotfish juveniles of about two to five months old were those schooling beneath FADs. Those specimens were planktivorous and, in their vast majority, sexually immature. The onset of gonadal maturity, however, which might be associated with higher energetic needs and hence different nutritional requirements, seems to trigger the shift of the fish to a new, more appropriate, habitat. This size-dependent change in habitat, related to either spawning requirements, and/or a change in the nutritional needs, as well as the early onset of the species' sexual maturity, along with its rather prolonged spawning period, considering also the fact that the pilotfish seems to be a rather short-lived species (RENONES *et al.*, 1999), might be mechanisms favouring its survival in the highly variable pelagic environment.

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