



NOTE

## Habitat selection by emperor fish larvae

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**ABSTRACT:** One of the great puzzles of coral reef fish ecology is how pelagic larvae locate the habitat in which they settle. The present study explored whether offshore emperor fish (Lethrinidae) larvae selected a specific reef and/or habitat at settlement. Although older juveniles are known to use back-reef seagrass beds as a potential nursery habitat, information is scarce regarding pre-settlement larvae. Using light traps anchored on the reef slopes at seagrass-replete and seagrass-free reefs (Ishigaki Island, Japan), lethrinid larvae were sampled weekly from April to June 2008. Larval abundance in light traps was similar on the 2 reef types. This result implies that lethrinid larvae did not or could not differentiate between the presence or absence of seagrass before settlement. However, in the laboratory, larvae collected from both reef types selected seagrass substrate rather than bare substrate or coral. In underwater visual censuses, newly settled juveniles were not observed on the seagrass-free reef, whereas they were abundant on the seagrass-replete reef. Together, these findings suggest that lethrinid larvae did not settle near the seagrass-free reef but rather preferentially settled near the seagrass-replete reef.

**KEY WORDS:** Settlement · Coral reef fish · Lethrinidae · Seagrass bed · Habitat selection

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### INTRODUCTION

Most coral reef fishes have an offshore pelagic larval stage, after which individuals move to nearshore habitats (e.g. coral reefs, seagrass beds and mangroves) where they enter the benthic juvenile stage (see review by Leis & McCormick 2002). One of the great puzzles of coral reef fish ecology is how pelagic larvae locate the habitat in which they eventually settle. At large spatial scales, some larvae detect the location of their settlement reefs by chemical cues emanating from the reefs (e.g. Atema et al. 2002, Huijbers et al. 2008) and/or reef noise (e.g. Montgomery et al. 2006, Simpson et al. 2008). Moreover, the swimming abilities of some reef fish taxa facilitate their reaching suitable post-larval habitats from long distances (e.g. Fisher et al. 2005, Irisson & Lecchini 2008). After reaching a reef, individuals choose a settlement habitat based on the presence or absence of specific benthic substrates,

conspecifics, and heterospecifics (e.g. Booth & Wellington 1998, Lecchini et al. 2005, 2007). However, little information is available on the ecological phase bridging the late oceanic and early benthic juvenile stages for coral reef fishes (see review by Leis & McCormick 2002). This lack of information may be due to the fact that larvae are generally minute and cryptic, and settlement typically occurs at night (Lecchini 2005).

Emperor fishes (family Lethrinidae) are widely distributed in the subtropical/tropical Indo-Pacific region (Carpenter & Allen 1989). *Lethrinus* spp. predominantly inhabit back-reef seagrass beds at the juvenile life stage (Wilson 1998, Nakamura & Tsuchiya 2008, Shibuno et al. 2008), thereby utilizing the beds as a potential nursery habitat that produces relatively more adult recruits per unit area than other juvenile habitats (Beck et al. 2001). Afterwards, juveniles move to coral reef habitats at maturity, and adults often form large aggregations for pelagic spawning at outer reefs. However, it is not yet

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fully understood how offshore lethrinid larvae locate their seagrass nursery habitat. Hypothetically, the settlement process from offshore to seagrass beds could be explained by: (1) offshore larvae detecting and directly moving to seagrass-replete reefs, or (2) larvae selecting seagrass beds after reaching a reef. In the former case, seagrass-replete reefs should attract offshore larvae, resulting in a possible abundance of newly settled juveniles on those reefs compared with seagrass-free reefs. In the latter case, larvae should colonize both seagrass-replete and seagrass-free reef types, those colonizing seagrass-free reefs delaying settlement until a suitable substrate is encountered.

The present study was designed to address the following questions, using *in situ* and *ex situ* experiments: (1) do lethrinid larvae have a preference for a seagrass substrate, and (2) do offshore lethrinid larvae select a seagrass-replete reef?

## MATERIALS AND METHODS

The present study was carried out on Itona and Tomino reefs at Ishigaki Island (24° 29' N, 124° 13' E), southern Ryukyu Islands, Japan (Fig. 1). The margin of each reef is 300 m from the shore. The coral-dominated area (hereafter the coral area), from 160 m from the shore to the reef edge, is comprised of primarily living and dead *Acropora* spp. and *Porites* spp. At Itona reef, the seagrass bed (11 ha) extends along the coast line between 30 and 120 m from the shore (0.5 to 1 m depth at low tide and 2 to 3 m depth at high tide), and is dominated by the seagrasses *Cymodocea serrulata*

and *Thalassia hemprichii* (110 to 130 shoots m<sup>-2</sup>; leaf height, 8 to 15 cm). The reef flat on Tomino reef (0.5 to 1 m depth at low tide, 2 to 3 m depth at high tide) is characterized by coral rubble and reef rocks lacking seagrass vegetation. No physical barriers exist between the coral areas and inner reef flats of each reef, and the distance between the 2 experimental reefs is 3 km. Water temperature (~25°C), salinity (~35), and underwater visibility (>15 m) did not differ between the 2 reefs during the study period.

At Ishigaki Island, lethrinids spawn from April to November, with peak reproduction between April and June (Ebisawa 1999) and subsequent settlement on Itona reef after about 20 to 30 d of pelagic larval development (Y. Nakamura unpubl. data). Although juvenile *Lethrinus* were easily identified to species level by their body color patterns, larvae and newly settled juveniles (<30 mm total length, TL) did not possess species-specific color patterns (semi-transparent body; McCormick et al. 2002). Therefore, all samples of *Lethrinus* spp. were treated as a single catch-all taxon in the present study.

Pre-settlement lethrinid larvae were sampled weekly from 14 April to 16 June 2008 using 4 light traps (Fig. 2) that were separated from one another by at least 50 m and anchored on the reef slopes (from 17:00 to 08:30 h) at Itona and Tomino reefs (Fig. 1). This observational study of larval abundance made use of only 2 study sites in a single year and was limited to showing general patterns on spatiotemporal scales.

Newly settled lethrinids (<30 mm TL) were surveyed in the back-reef habitat at each site using an underwater visual belt transect survey method. Surveys were conducted along 10 belt transects (30 × 1 m) on the same day or 1 d after each light trap sampling event. Counting was performed on seagrass beds and on unvegetated reef flats at Itona and Tomino, respectively (Fig. 1).

To evaluate seagrass preference in lethrinid larvae, 2 laboratory habitat choice experiments were performed using larvae collected at Tomino and Itona reefs. Expt 1 was designed to test whether a seagrass substrate (coral absent) would attract lethrinid larvae, whereas Expt 2 tested whether these larvae would select a seagrass rather than a coral substrate. The 2 experiments were performed in an indoor facility under dark conditions (isolated from external sources of light and sound) during the evenings (17:30 to 22:30 h) within 24 h after larval capture. A cylindrical black PVC tank was used for the choice experiments (Fig. 3). In Expt 1, 8 mm PVC

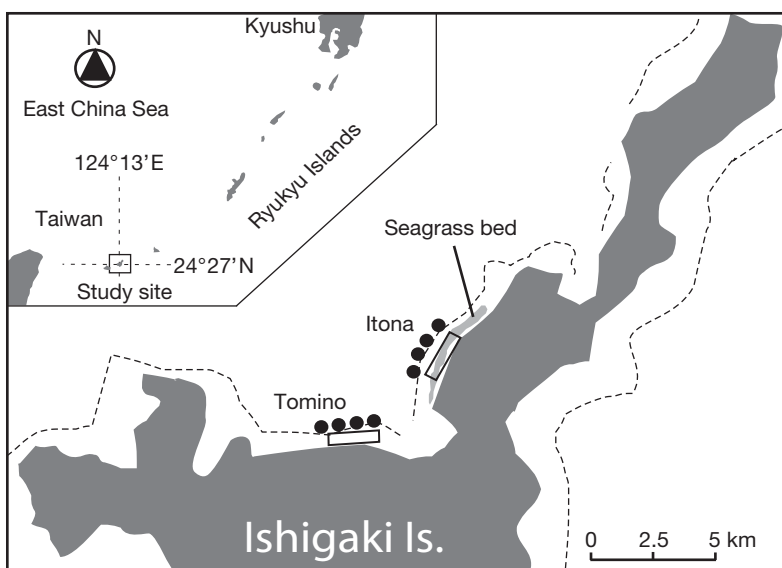


Fig. 1. Itona and Tomino reefs, Ishigaki Island, Japan. Dashed line indicates reef margin. (●): light trap sampling station; (□): visual transect survey station

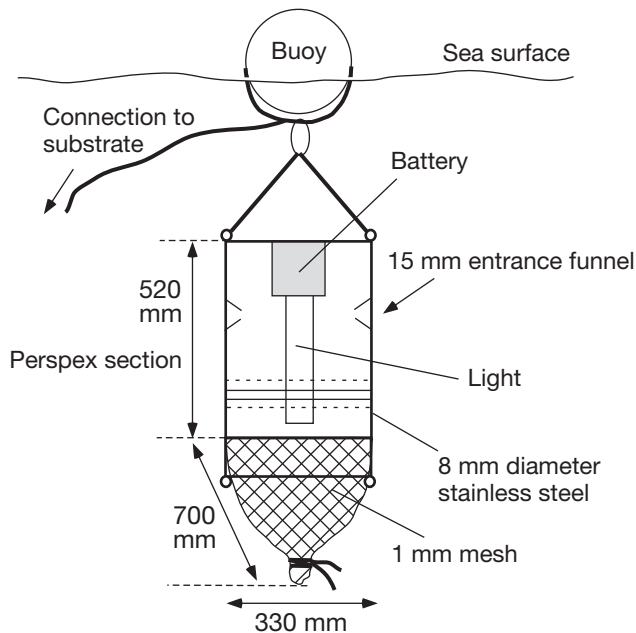


Fig. 2. Diagram of light trap. Fish larvae entered the perspex section (protected by a stainless steel frame to which a buoy was attached) via 4 entrance funnels, being attracted by a 6 W fluorescent lamp (National BF-8951). Four 1.5 V alkaline batteries powered the light source, lasting for up to 10 h. The entrance openings were in the top 1 m of the water column. The collection mesh permitted substantial water movement, which helped maintain the fish larvae in good condition. Trapped larvae were placed in buckets of aerated seawater and transported to the laboratory

mesh (600 cm<sup>3</sup>) with seagrass *Cymodocea serrulata* shoots (50 shoots) and PVC mesh without *C. serrulata* shoots were placed on opposite sides of each tank (n = 3 tanks). In Expt 2, 8 mm PVC mesh with either *C. serrulata* shoots or branching coral (*Acropora* spp.) were placed on opposite sides of each tank (n = 3 tanks).

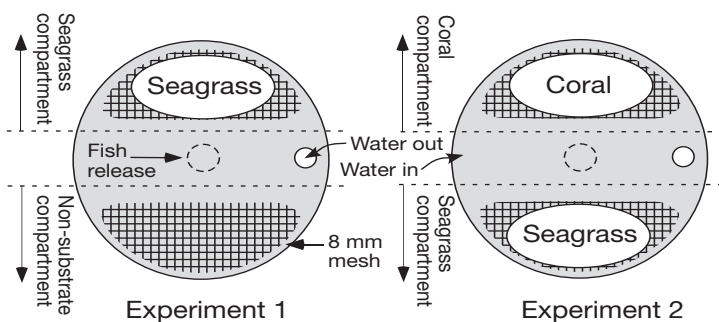


Fig. 3. Design of the aquarium system used to explore the habitat preference of settlement-stage lehrinid larvae. The cylindrical black PVC tank measured 64 cm in diameter and 46 cm in height, and contained 70 l when filled to the overflow outlet, about 15 cm below the rim. UV-sterilized seawater flowed into the top of the tank at 0.7 l min<sup>-1</sup> to ensure a continuous supply of fresh seawater

Both seagrass and corals were examined to confirm the absence of animals such as sound-emitting shrimps (Montgomery et al. 2006). A single larva was released in the center of each tank, and after 30 min the side of the tank occupied by the larva was recorded. A total of 9 larvae (collected on 26 May 2008) were used from each of the 2 sites (n = 18 per experiment), and 3 trials were performed in each of the 3 tanks for the 9 larvae from each site. All specimens were used only once. For each trial, each habitat (seagrass and coral) was randomly reassigned to the eastern and western sides of the tank. The observed frequencies of habitat choice within an experiment were compared with the binomial probability of observing such an outcome. A 2-tailed binomial probability of less than 0.05 was interpreted as a statistically significant habitat preference.

## RESULTS AND DISCUSSION

During the sampling period, lehrinid larvae colonized both Tomino and Itona reefs on 6, 26 and 29 May and 13 June (Fig. 4a). Two-way repeated measures ANOVA with the factors of reef type and date showed a significant difference in larval abundance between sampling dates (n = 7,  $F = 7.505$ ,  $p < 0.01$ ), but no significant differences between reefs ( $F = 0.002$ ,  $p = 0.9$ ) or their interaction ( $F = 0.630$ ,  $p = 0.7$ ). The underwater visual surveys at Itona reef showed the presence of newly settled lehrinid juveniles (25 to 30 mm TL) in seagrass beds, most settlement occurring from the end of May to early June (Fig. 4b). Larger-sized juveniles (30 to 40 mm TL) were also found in seagrass beds in mid-June, and included *Lethrinus atkinsoni*, *L. harak*, *L. obsoletus*, *L. genivittatus* and *L. ornatus*. Although many lehrinid larvae occurred at Tomino reef, we did not observe newly settled juveniles on that reef (Fig. 4b). In the laboratory experiments, larvae from both Tomino and Itona reefs showed a significant preference for the seagrass substrate, most larvae staying in the seagrass compartment of the aquarium in both experiments (Table 1).

Although the low spatial and temporal replication of the present study limits the strength of inference drawn from our results, the colonization patterns of lehrinid larvae on a seagrass-free reef indicated that they might not use seagrass-derived olfactory cues for orientation with respect to the settlement reef. Recently, Arvedlund & Takemura (2006) found that *Lethrinus nebulosus* settlers that were reared in captivity selected seagrasses using olfactory cues. Our laboratory experiments also showed a signifi-

Table 1. Binomial tests for seagrass substrate preference in late-stage larvae from 2 reef states: seagrass-replete (Itona) and seagrass-free (Tomino). A single larva was released in the center of experimental tank, and after 30 min the side of the tank (seagrass or non-substrate in Expt. 1 and seagrass or coral in Expt. 2) occupied by the larva was recorded. Data correspond to number of larvae per compartment (seagrass, non-substrate, coral) in 9 experimental trials

Larval type	Expt. 1			Expt. 2		
	Seagrass	Non-substrate	p	Seagrass	Coral	p
Itona larvae	9	0	<0.01	7	2	0.18
Tomino larvae	8	1	0.04	8	1	0.04

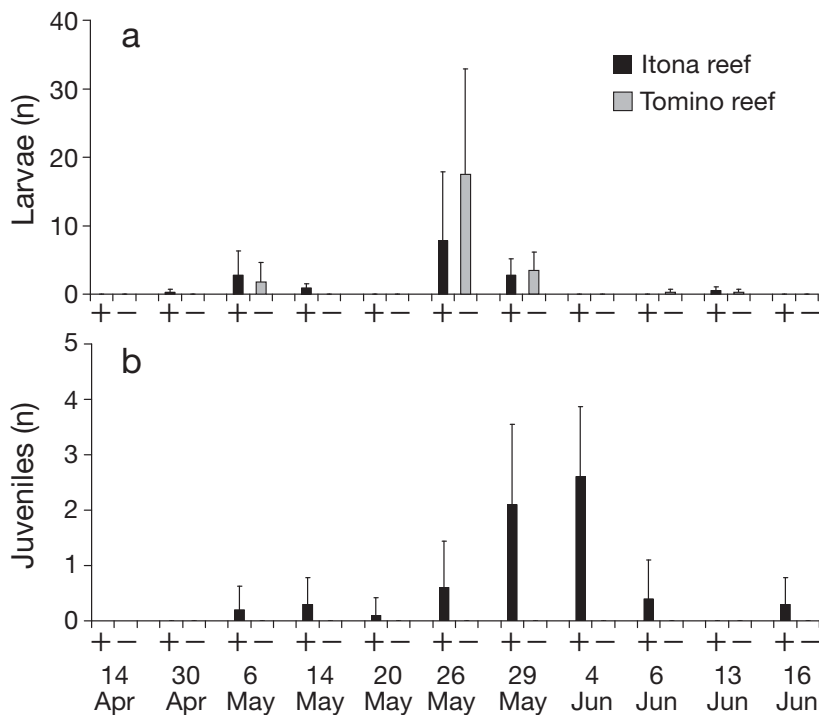


Fig. 4. Mean (+SD) abundance of lethrinid (a) larvae per light trap (one night,  $n = 4$ ) and (b) juveniles per transect ( $30 \text{ m}^2$ ,  $n = 10$ ) at Itona (+) and Tomino (-) reefs during the study period. Days represent the dates of light trap sampling

cant preference for seagrass by lethrinid larvae. In the aquaria, only seagrass chemical cues were present, whereas the ocean environment includes many chemical cues from various organisms (e.g. macroalgae, corals, crustaceans and mollusks) (Paul et al. 2006), possibly nullifying any chemical cues from the seagrasses (but see Huijbers et al. 2008). On the other hand, chemical odor plumes containing seagrass extracts could diffuse over a fairly wide area, including a significant distance offshore, thereby helping actively swimming larvae to home in on areas generally suitable for settlement. For example, during low tide, chemically-rich water exits the lagoon, providing a strong chemical signal up to 4 km from the island (Atema et al. 2002). Given that the larvae did not dis-

criminate offshore as to whether or not a reef contained seagrass (at a scale of <5 km), larvae would be supplied from offshore to nearshore areas at both Tomino and Itona reefs. However, such supply might not always be the case at tropical coasts because lethrinid juveniles were often found in small-scale seagrass beds; i.e. slight seagrass odors would not guide offshore larvae toward the latter seagrass beds. Nevertheless, further study of multiple sets of seagrass-replete vs. seagrass-free reefs at varying distances from the seagrass-replete reefs (up to 10–20 km away) would be needed to identify the importance of seagrass odors (and sound) as a cue for orientation with respect to the settlement reef.

The underwater visual surveys showed the presence of newly settled lethrinid juveniles in seagrass beds at Itona but not Tomino reef (Fig. 4b). The absence of juveniles on Tomino reef, although this reef was colonized by larvae, could have been due to delayed settlement in the absence of a suitable settlement substratum or high post-settlement mortality. Several studies showed that predation removed between 30 and 90% of new settlers within the first weeks of settlement (see review by Doherty 2002). Moreover, food availability may influence the survival of lethrinid juveniles, with higher mortalities in unvegetated areas. However, even if post-settlement mortality was high in non-juvenile habitats (Tomino reef), if larvae settled on the latter reef, we would not overlook newly settled juveniles. This

is because the visual surveys were conducted immediately after the larval sampling events, and we could always count newly settled individuals since the maximum number of post-settlement days was estimated as less than 3 to 4 based on the main pigment change of larvae (72 h; McCormick et al. 2002). Therefore, habitat choice at settlement seems likely to account for the differences in lethrinid settlement patterns between the sites, although several factors could influence the settlement patterns. Overall, our results suggest that when lethrinid larvae arrived at seagrass-free reefs, they failed to settle and possibly continued their search for seagrass-replete reefs.

In conclusion, the present study showed that lethrinid larvae did not or could not select their settlement reefs, despite having a preference for a specific sub-

strate, but might locate their settlement habitats after reaching a reef. However, the conclusions of the present study must be interpreted with caution as it was conducted at a single location in a single year, using a catch-all taxon approach, thus restricting the spatial and temporal replication required for general conclusions.

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