SHORT COMMUNICATION

Prey selectivity in piscivorous bluefin tuna larvae reared in the laboratory

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Prey size selectivity in piscivorous fish larvae is important to both aquaculture and fisheries science, but laboratory experiments are few. We analyzed selective foraging in Atlantic bluefin tuna larvae (*Thunnus thynnus*) using two larval fish prey species. The experiments revealed that selective foraging of prey sizes differed among bluefin tuna predator sizes (15–25 mm SL) and prey species, bonito (*Sarda sarda*) and seabream (*Sparus aurata*). The observed pattern suggest a general preference for small bonito prey larvae but large seabream prey. Thus, prey size alone is not the only trait responsible for size selectivity in piscivorous fish larvae.

KEYWORDS: bluefin tuna; *Thunnus thynnus*; piscivory; fish larvae; prey; predator; selectivity; diet

The larval phase is the developmental stage with the highest rate of change in size-dependent processes. Predator–prey analyses in zooplanktivorous fish larvae show that larger prey are usually selected as larval size increase (Brooks and Dodson, 1965; Bremigan and Stein, 1994; Seljeset *et al.*, 2010). Prey size variability as larvae develop is also a requirement in larviculture that is usually facilitated by changing prey species over time, e.g. changing diet from rotifers (*Brachionus plicatilis*) to brine shrimp (*Artemia salina* instar II). Most large predatory fish species are piscivorous during their larval stage (Llopiz, 2013), yet larval prey size selectivity is unknown because prey fish larvae from stomachs of larval predators from field samples are difficult to identify and measure (Folkvord, 1993).

The main objective of this study is to assess the selective predatory behavior at different predator sizes in piscivorous Atlantic bluefin tuna (*Thunnus thynnus*) larvae reared in the laboratory using two prey species, bonito (*Sarda sarda*) and seabream (*Sparus aurata*), the latter currently used for larviculture (e.g. Reglero *et al.*, 2014). These two prey species are good examples of the general larval prey morphological types and larval developmental strategies that coexist with Atlantic bluefin tuna larvae (Torres *et al.*, 2011). Therefore, our experiments aimed to advance understanding of predator–prey relationships during the piscivorous larval stage of bluefin tuna. Additionally, the results can be used to develop techniques for the larviculture of the Atlantic bluefin tuna.

Cultures of larvae of the three species (predator and prey) were held in parallel at the Institute of Marine Oceanography (IEO) facilities in Mazarrón (SE Spain). Batches of fertilized bluefin tuna eggs were obtained from naturally spawning captive adult tuna in the farming facilities of Caladeros del Mediterráneo SL at El Gorguel, Cartagena (SE Spain). The bluefin tuna eggs were collected and transported to IEO where the experiments took part. Successive groups of eggs of bonito and seabream were obtained almost daily from captive broodstocks at the IEO during the study period.

Fertilized eggs of seabream and bonito were incubated separately in 400 L tanks and those of bluefin tuna either in 1500 or 5000 L tanks. Incubation was carried out with an upwelling current (8–10 renewals per day), mild aeration and continuous photoperiod with light intensity close to 300 lux. The incubation temperature for seabream eggs varied between 21 and 23°C, for bonito 22 and 24°C and for bluefin tuna eggs 21 and 26°C. The larvae of bonito and seabream remained in the 400 L tanks during the whole yolk-sac larval stage until 2 days post hatching (dph). Then, the larvae were moved to 1500 L cylindrical tanks until the beginning of the experiments. Bluefin tuna larvae remained in the 1500–5000 L tanks until the beginning of the experiments.

The initial larval stocking densities in the 1500 or 5000 L tanks were ~50–100 larvae L^{-1} for seabream, 20–40 larvae L^{-1} for bonito and 10 larvae L^{-1} for bluefin tuna. All larvae were fed with live prey supplied in excess twice every day. The feeding schedule consisted of enriched rotifers (*Brachionus plicatilis*) from 2–12, 2–15 and 2–18 dph for bonito, seabream and bluefin tuna, respectively, with densities within the tanks maintained at 10 rotifers/mL. Enriched Artemia (*Artemia salina* instar II) was added four times a day from 6, 13 and 16 dph onwards for bonito, seabream and bluefin tuna, respectively. We added 10 or 30 mL of paste of concentrated *Chlorella* (Super fresh Chlorella SV-12, Chlorella Industry Co., Ltd., Japan) four times per day in every 1500 or 5000 L tank. Additionally,

cultivated microalgae (*Nannochloropsis gaditana*) were added twice per day until 18 dph to bluefin tuna cultures.

Once bluefin tuna larvae began the piscivorous diet (18 dph onwards), they were placed individually in 8 L transparent plastic tanks during the late evening and left during 12 h in darkness without food for acclimation at 25°C. Early in the morning, the light was switched on and individual bluefin tuna larvae were simultaneously offered 40 small yolk sac larval preys (YSL) and 40 large larval prey of either seabream or bonito larvae. The small larval prey size corresponded to 1–2 dph YSL collected directly from incubators. The average body length ($\pm$ SD) measured as standard length of the small YSL bonito prey was 4.2 mm ($\pm$0.3, $n_{measured} = 61$) and the YSL seabream 3.4 mm ($\pm$0.04, $n_{measured} = 10$). Due to the different growth rate of bonito and seabream, we used 7–10 dph bonito as large prey (average SL = 6.4 mm $\pm$1, $n_{measured} = 72$) and 20–25 dph seabream as large prey (average SL = 5.8 mm $\pm$ 0.8, $n_{measured} = 20$). In each trial, the bluefin tuna larvae were offered the larval prey during 3 h or until they had ingested around 50% of the prey items ensuring prey of both types were left in the tank at the end of the experiment.

We repeated the trial twice using the same larvae and then the larvae were sampled. The time elapsed between trials during the same day was 3 h to ensure the evacuation of the stomachs between trials (Young and Davis, 1990; unpublished personal results). Three size groups of bluefin tuna larval predators were used, 15, 20 and 25 mm body length measured as average standard length, all of them in the post-flexion developmental stage. The number of trials for the three different sizes of predator bluefin tuna larvae (15, 20 and 25 mm SL) were 33, 30 and 18 when offered bonito and 27, 28 and 15 when offered seabream larval prey. Variability in the number of trials across larval sizes is due to reduced availability of large bluefin larval size classes. We estimated the Chesson's selectivity index (α, Chesson, 1983) to determine prey size selectivity. Random feeding was tested using a Student *t*-test to compare the estimated selectivity index to 0.5 (two food categories).

The results suggest that bluefin tuna larvae are very selective and patterns of selectivity are different depending on the species of larval prey offered and the larval predator size (Fig. 1a and b). When feeding on bonito, small prey were positively selected ($\alpha > 0.5$) compared with the large prey ($\alpha < 0.5$) by the larval predators 15 and 20 mm body length (*t*-test: mean α versus 0.5, $P \leq 0.005$ for both predator larval sizes), whereas the largest predator larvae (25 mm) ingested both small and large prey (*t*-test: mean α versus 0.5, $P = 0.07$; Fig. 1a). Larval predators of 15 mm did not show prey selective behavior significantly different from random feeding when feeding on
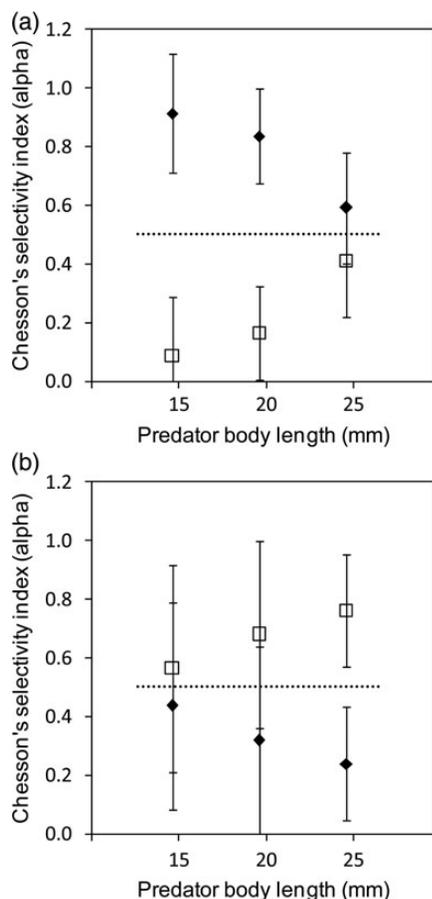
Fig. 1. Mean selectivity of bluefin tuna larvae of the three sizes feeding on YSL (black rhomboid) and large prey (white squares) of (**a**) bonito and (**b**) seabream. Random feeding is shown by the horizontal line at 0.5. Chesson's index value above 0.5 indicates positive selection, whereas values below 0.5 represent negative selection. Standard deviations are shown in the vertical bars.

seabream (15 mm, t-test: mean α versus 0.5, $P = 0.28$), whereas large seabream prey was positively selected ($\alpha > 0.5$) compared with small prey ($\alpha < 0.5$) for the largest larval predators (20 and 25 mm, t-test: mean α versus 0.5, $P \leq 0.005$; Fig. 1b).

This experiment provided a choice between two prey alternatives and suggests prey size selective foraging during the piscivorous stage of bluefin tuna larvae. To our knowledge, this is the first study of selective foraging of alternative larval prey in piscivorous fish larvae and it shows how both size and prey-type can make a difference in the diets. In piscivores, selective foraging is often related to the ingestion of the largest of the suitable prey (e.g. Mittelbach and Persson, 1998). However, depending on the availability of alternative prey, it may be profitable to actively select the small-sized prey, a strategy that can maximize energy intake per unit of time when encounter rates, handling times and capture success between prey

sizes differ (e.g. Turesson *et al.*, 2002; Visser and Fiksen, 2013).

When offered the choice between equally abundant small and large prey, larval predator response differed depending on the larval prey species and the predators' size. The observed patterns could results from non-selective foraging just because of differences in capture success among prey sizes. Alternatively, bluefin predators may make an active behavioral decision to ignore prey with low success rate, which may maximize food intake rate and involve a "real" selective foraging behavior (Stephens *et al.*, 2007). Although we did not measure actual attack rates, we observed bluefin tuna tried to attack the "large bonito prey" but after a few attacks with low success rate they did not attack them anymore. In fact, bluefin tuna larvae modified their swimming speeds to try the best way to attack the large bonito prey that may swim fast. In a sense, they gradually became more selective through a process of learning by not wasting time trying again after a few trials without success. Therefore, we believe our results reveal true selective foraging behavior. In contrast, they gradually attack larger seabream prey and due to the high success rate large size seabream are further incorporated in the diet as bluefin tuna larvae grow.

Size is an important factor that influences many biological processes (Miller *et al.*, 1988), but size-based analyses alone are not enough to understand foraging behavior in piscivorous larvae. Bonito have a tadpole like morphology and a big mouth gape that facilitate piscivory during the larval stage. Thus, according to optimal diet theory (Stephens and Krebs, 1986; Stephens *et al.*, 2007; Visser and Fiksen, 2013), selecting the small bonito prey could reward higher profitability if handling times were lower or capture success higher than for the largest bonito prey. In contrast, larger bluefin predators could incorporate large prey in their diet because of better foraging abilities. Seabream larvae may be comparatively slower swimmers than bonito (personal observation), with a more elongated body shape maintaining a planktivorous diet over their larval development. Large predators may decide not to include small prey in their diet and waste valuable search time if there are more profitable prey available (Visser and Fiksen, 2013).

Bluefin tuna larval predators can react to larval prey-size/developmental stage structured fields modifying their foraging behavior by selecting different prey to be included in the diet. Larval size variability is closely linked to the larval developmental stage. In nature, we expect different developmental stages and sizes of larvae overlapping in time and space (Torres *et al.*, 2011). We expect similar mechanisms in most apex predators with a piscivorous phase during the larval stage. Further behavioral measurements in the future will help accurately

describe the process of selective foraging behavior in bluefin tuna larvae.

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