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Research Article

Feeding Behaviour of Larval European Sea Bass *(Dicentrarchus labrax L.)* in Relation to Temperature and Prey Density

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Abstract

The feeding behaviour of larval European sea bass (*Dicentrarchus labrax*, L.) was analysed in relation to temperature and prey density under controlled laboratory conditions with the aim to assess the ability of larval fish to change the feeding tactic as a response to environmental changes. Larvae were acclimated for 20 days at three different temperatures (19, 22 and 26°C), and their feeding behaviour was then video-recorded in experimental trials, at two prey densities, consisting of swarms of 400/l and 1440/l *Artemia* nauplii. Results showed that there was a significant effect of the interaction between temperature and prey density on the proportion of swimming activity that was reduced at the high temperature-high prey density combination. This suggested a switching in the larval feeding behaviour from an active to an ambush tactic, when the temperature reached 26°C and the prey density was 1440 /l *Artemia* nauplii. These results are consistent with the current literature on fish larval behaviour in showing that the foraging tactic can be modulated by the interaction of different abiotic and biotic factors characterising the rearing environment.

Keywords: Behavioural Response; Temperature; Climate Change; Larval Feeding; European Sea Bass

Introduction

The study of environmental factor effects on the behavioural performance of larval fish could be useful in aquaculture context, since these factors may in turn influence both growth and survival. The foraging ecology of juvenile fish is strongly influenced by development, with the consequent effects on the increase of gape size, by swimming performance, and by a number of abiotic and biotic factors such as temperature, turbidity, turbulence, prey density, light characteristics, and by their potential interactions [1-6]. The European sea bass (*Dicentrarchus labrax* L.) is a widely cultured fish in the Mediterranean region and a large number of larvae are produced by means of different techniques. As regards the larval stage of this species, the feeding performance of individuals has been analyzed in relation to ontogeny, light intensity and prey density, showing that prey density and light characteristics modulate the larval feeding behaviour [5, 7]. Recently, the effects of temperature increase on some behavioural patterns have been investigated in sub-adult and adult individuals of *D. labrax* [8, 9]. Considering this background of knowledge and the economic importance of this species, the European sea bass may represent an interesting and useful study model to investigate the effects of the interaction among abiotic factors on the behavioural performance of a commercially important fish. In the present paper, the feeding performance of larval European sea bass is analyzed under laboratory conditions as a response to increasing temperature that may affect fish metabolism and prey density that is known to be able to modulate the feeding behaviour of this species [7]. In visual zooplanktivorous fish predators, prey density plays a crucial role, as feeding frequencies change in relation to different prey densities, and attack success can be reduced by increase in prey group size [10, 11]. The overall feeding performance of a fish could therefore not only be affected by temperature, as a consequence of increased metabolism due to global warming, but also by the potential concomitant variation of prey availability in the environment.

In the present work, the effects of a 20 day-long acclimation to three temperature regimes (19, 22 and 26°C) were tested on the feeding behaviour of three-month-old hatchery-reared European sea bass larvae, in relation to two different prey (*Artemia* nauplii) densities, under controlled laboratory conditions. The choice of the three experimental temperatures was made on the basis of the mean water temperatures in the Venice lagoon during the months of April and May that represent the ascent phase of larval and juvenile fish of European sea bass to the coastal and lagoon habitats [12], so they were selected within the tolerance limits of the species. The aim of the present study was the assessment of the potential ability of larval fish to switch their feeding tactic as a response to a potential environmental change.

Material and Methods

Fish housing and maintenance

A stock of 500 European sea bass larvae, about 3 months old, was obtained from a professional hatchery (Agroittica Toscana srl) during April 2012. The mean body size of these fish was around 15 mm, so slightly below the mean size corresponding to the metamorphic transition [13, 14]. Since this size is closed to the ontogenetic transition from the larval to the juvenile phase, it is critical for the study of the effects of environmental factors on the larval behavioural performance that may affect subsequent growth and survival. Larvae were randomly distributed with equal final density (1 larva/L) into 3 tanks of 190 l capacity (designated as "community tanks"), each filled with artificial sea water of 30%, and maintained at ambient temperature (19°C), replicating the housing conditions of the hatchery that provided the fish. The same chemical–physical conditions experienced by fish in the source hatchery were maintained for the first 10 days to reduce the potential stress level of the fish. Successively, temperature was raised by means of aquarium heaters (Prodac thermal, cittadella, Pd, Italy), connected with thermostat (Hydor, hydroset T03200, Bassano del Grappa, VI, Italy), with a progressive increase of 1°C each day to the final experimental temperature of 19°C, 22°C, and 26°C and then maintained for 20 days. Larvae were fed with *Artemia salina* nauplii *ad libitum*. The photoperiod was maintained at 12:12 LD.

Experimental set-up and procedure

Experiments were performed in small tanks (30X18X20 cm) designated "experimental tanks". The lateral and posterior walls were covered with thick, and opaque blue papers, to reduce visual disturbance. Light intensity was 1200/1300 lx, measured at the water surface with a digital luximeter (MITEK, MK5334). The feeding behaviour of *D. labrax* larvae was analyzed in the presence of two different prey densities, consisting of swarms of 400/l (D1) and 1440/l (D2) *Artemia* nauplii. The two different levels of prey density were determined by means of preliminary feeding trials. These densities were not intended to simulate natural conditions, but to create a marked difference in the conditions of the predator visual fields. The aim was to test for the potential predator ability to adjust their feeding behavior in relation to rarefied (D1) and highly dense (D2) swarms of prey under different temperature conditions. After the acclimation period, a larval fish was haphazardly captured from a "community tank" and transferred to the "experimental tank", filled with the same water of the "community tank" that corresponded to one of the three thermal treatments. After 10 minutes of acclimation in the "experimental tank", a prey-containing solution was rapidly released into the "experimental tank" to obtain one of the two established final densities (Figure 1). The water temperature in the

"experimental tank" remained constant for the whole duration of the experiment.

labrax L.) while searching for Artemia nauplii prey.

Subsequently, a digital video-camera (Canon camcorder legria HF S30, Tokyo, Japan) was focused on the fish, in front of the tank, and switched on to follow and record the larval behaviour for the following 10 minutes. After the completion of each 10 min recording, every tested fish was measured for their Standard length, by means of a calliper (± 0.1 mm). Then both fish and water were replaced, and the subsequent recording was performed in the same "experimental tank". Fish used for the trials were transferred to new "community tanks", so that each fish was never used twice for the experimental trials. The temperature and prey density combination used for each trial was randomly determined. On the whole, 44 larvae were tested, with 6 to 8 larvae being tested for each temperature/prey density combination.

Behavioural and statistical analyses

The following behavioural responses were measured. Latency (LA): time spent by the larval fish (s) to restore the ordinary swimming activity, after the introduction of preys, meaning time passing from the introduction of preys, when an unavoidable fear reaction is elicited in fish, to the time when the larval fish started to swim for prey searching; Swimming activity (SA): time spent in ordinary swimming, being the complementary measure of this variable the time spent motionless (% time, at the net of latency time); Attacks (FA): i.e. rapid burst of swimming directed to the prey, and in many cases culminating with the prey ingestion (frequency, n m^{-1}). Data were log-transformed to meet the assumptions of data normalisation and homogeneity of variances, allowing the use of parametric statistics. Log-transformed individual behavioural variables were correlated with Standard length, to check for a possible effect of body size. Then, the combined effects of temperature and prey density were tested by means of two-way ANOVA, performed on each log-transformed behavioural variable.

Results

Mean body size (SL) of the experimental fish was 15.99 cm, ranging from 13.17 to 20.78 cm, and there was no statistically significant correlation between log transformed body size and any of the log transformed behavioural variable (Pearson Correlation, Log SA, r^2= -0.04, p>0.05; Log FA r^2 = 0.2 p>0.05; Log LA r^2 = 0.17 p>0.05). Average values of the three behavioural variables did not show marked changes neither across temperature, nor between prey density (Table 1), with the exception of the proportion of swimming activity that, at 26°C, decreased from an average of about 83 % at D1 to an average of less that 50% at D2 (Table 1).

	19 ° C		22 ° C		26 ° C	
	D1 (8)	D2 (8)	D1 (6)	D2 (8)	D1 (6)	D2 (8)
Latency (s)	24.8 ± 10.1	16.4 ± 12.3	13.3 ± 9.8	18.1 ± 10.1	34.2 ± 48.7	43.5 ± 57. 5
Swimming activity (%)	84.3 ± 10.3	91.8 ± 5.5	88.3 ± 10.5	81.6 ± 18.2	83.2 ± 17.4	47.6 ± 31.8
Attacks (n m^{-1})	8.7 ± 2.8	5.5 ± 1.7	9.1 ± 3.8	7.3 ± 3.5	10.2 ± 5.1	11.18 ± 5.2

Table 1. Means ± s.d. of the three behavioural responses measured, at each combination of prey density and temperature. D1= low prey density, D2=high prey density. In parentheses, the number of larvae tested for each combination.

Two-way ANOVA did not reveal any statistically significant effect of temperature and prey density on Log FA and Log LA (Table 2), and there was a significant effect of the interaction between Temperature and Prey density (Table 2). In particular, there was a sharp decrease of swimming activity at the 26°C/D2 treatment that corresponded to the highest temperature/high density treatment (Figure 2).

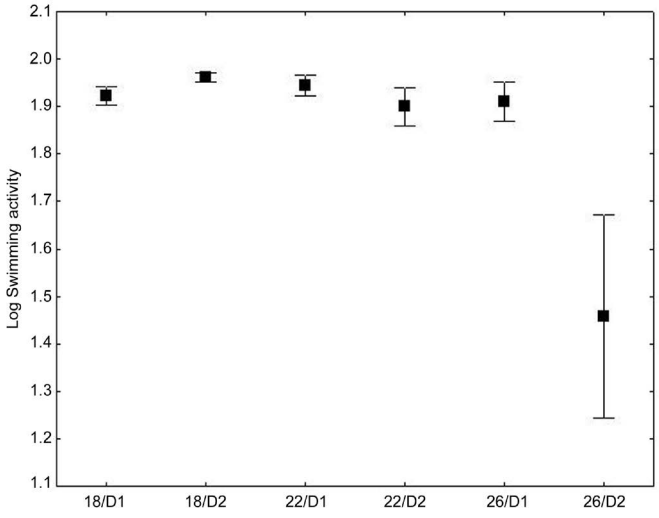

Figure 2. Means ± s.e. of log transformed proportion of swimming activity at each experimental treatment.

	LA				SA				FA			
	SS	DF	F	P	SS	DF	F	P	SS	DF	F	P
T	0.31	2	1.24	0.30	0.58	2	4.06	0.06	0.22	2	2.49	0.09
PD	0.008	1	0.06	0.79	0.25	1	3.5	0.025*	0.11	1	2.45	0.125
T*PD	0.39	2	1.55	0.22	0.49	2	3.46	0.04*	0.14	2	1.58	0.22
Error	4.82	38			2.72	38						

Table 2. Results of two-way ANOVA, applied to the three measured behavioural responses, using Temperature (T) and prey density (PD) as categorical predictors (on log-transformed data). LA= latency, SA= swimming activity, FA= frequency of attacks; * significant at $p< 0.05$.

Discussion

The present study aimed to analyze the combined effect of environmental temperature and prey density on the feeding behaviour of larval European sea bass by evaluating three different variables as latency, swimming activity and attacks. Overall, the results suggested that in the tested range (19 to 26°C and 400 *Artemia*/l versus 1440 *Artemia*/l), prey density and temperature only interact at the combined level 26°C – 1440 *Artemia*/l affecting the proportion of swimming activity but not the latency, nor the attack frequency of fish larvae. Despite the high data variability, due probably to the low sample size used for each density/temperature combination, this difference was statistically significant and the magnitude of the difference was remarkable, with a decrease in the proportion of swimming activity at the high prey density/high temperature combination of about 40 %. According to the current literature, swimming activity generally tends to increase with larval size especially in relation to the development of fins and to the improved vision [2, 6, 15]. Nevertheless, in our study no correlation between body size and behavioural variables was found, probably in relation also with the small body size range of the larvae used in our trials. Our results showed a drastical decrease in larvae swimming activity when temperature reached 26°C and *Artemia* density 1440/l. Moreover, the variation in the proportion of swimming activity was also higher at this prey density/temperature combination. A possible explanation of our results is that the combination of high prey density and high temperature determines the tendency to change the larval feeding tactic, switching from an active to an ambush predation tactic, as observed by Killen et al. [16]. In terms of effects of water temperature, a number of experimental studies conducted on the European sea bass showed that the optimal temperature for the swimming performance is around 24°C and that the swimming speed and performance tend to decrease above this temperature [17-19]. This is consistent with the reduction of swimming activity observed in our 26°C treatment, especially when this effect is enhanced by the concomitant effect of increased prey density. By contrast, the other two components of the feeding tactic analyzed, that is latency to attack and frequency of attacks, did not show any clear pattern of variation in relation to temperature and prey density. Our results are consistent with other experimental studies on fish larval behaviour showing that the swimming activity can be influenced by the interactions among different environmental factors, such as temperature, turbulence and light [2,3,15]. In conclusion, the present study suggests an interactive effect of temperature and prey density on the feeding behaviour of European sea bass larvae. These results, together with those previously obtained [5, 7], show a high influence of the rearing characteristics such as lighting regime, ontogeny and prey density on the feeding performance of European sea bass larvae that may potentially affect larval growth and survival. Thus, these studies provide suggestions that may help the optimisation of rearing conditions in the context of European sea bass aquaculture.

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