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INFLUENCE OF DIFFERENT ALGAL DIETS ON LARVAL GROWTH RATES IN THE MARINE SERPULIDAE POLYCHAETE WORM *Spirobranchus kraussii*

Fatemeh Lavajoo*

Department of Marine Biology, Faculty of Marine Science and Technology, University of Hormozgan, Iran

*Corresponding Author, Email: f.lavajoo@gmail.com

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ABSTRACT

Effects of food availability on larval growth and survival of *Spirobranchus kraussii* were studied by feeding larvae different algal diets. Newly hatched larvae of *S. kraussii* were fed four different marine microalgae species, singly and in various mixtures. The best growth was observed when fed *C. vulgaris*, *N. oculata* as a single species and mixed-algal diet during day 15 after fertilization. Mortality was low for larvae (max. 5%); survival rate more than 95%. These results suggest that *S. kraussii* larvae have the capacity to feed using alternative sources of energy, and food size and quality can affect their growth and sustainability.

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INTRODUCTION

About 90000 species of marine benthic invertebrates produce a meroplankton larva as part of their life in the water column (Thorson, 1964; Almeda et al., 2009; Young et al., 2011), and significant dispersal can only happen during this free-swimming larval stage of benthic invertebrate species (Strathmann, 1990; Levin and Bridges, 1995; Pechenik et al., 2010). In population dynamic planktonic larvae, some factors such as natural mortality, dispersion, feed and development time may impact the permanence of larva and population recruitments (Blanner, 1982; Pedersen et al., 2010). Physical and biological processes can alter planktotrophic larval development and dispersal (Cowen and Sponaugle, 2009). The concentration of suspended food particles such as phytoplankton may affect feeding opportunities for planktotrophic invertebrate larvae in coastal habitats (Mackas et al., 1985; Pitcher et al., 1991; Franks, 1992, Litaker et al., 1993; Qiu and Quian, 1997). After hatching, planktotrophic larvae must feed on phytoplankton for days or weeks before reaching the metamorphosis

stage (Pechenik and Tyrell, 2015). In the meantime, some factors such as the presence of predators or food can affect successful development of the species (Eno et al., 1997; Hawkins et al., 2003). Inadequate food concentrations during larval development may impact the metamorphosis stage and prolong this stage (latent effect; reviewed by Pechenik, 2006). The species of phytoplankton can also alter larval growth rates when used as larval food (Pechenik and Fisher, 1979; Klinzing and Pechenik, 2000). The research confirms that climate change will have a huge impact on plankton, but scientists still do not know how exactly that impact will look.

The Persian Gulf is a relatively shallow body of water and the extreme salinity and temperature fluctuations of its waters have created unique marine and coastal ecosystems (Bayani, 2016). *Spirobranchus kraussii* (Baired, 1865) is a Serpulidae polychaetae that has been recently found and covers a vast surface of rocky shores in Bandar Abbas (Persian Gulf, Iran) (Lavajoo and Amrollahi, 2015). *S. kraussii* species are easily recognizable by several characteristics such as their calcareous tube, operculum, colorful radiolar crown, chaeta and uncini. Pores between their tubes gather water and serve species of predators and also act as a breeding place and nursery area for coastal organisms (Vinn, 2013; Sanfilippo et al., 2017). However, this species is a fouling organism, but it has an important ecological role in the lives of other organisms living on the rocky shores of Bandar Abbas (Persian Gulf, Iran), and any changes in the Persian Gulf basin may have an impact on the life cycle of marine ecosystems. The effect of a varied algal diet on the larval development of many invertebrates has been studied (Gosselin and Sewell, 2013; Pechenik and Tyrell, 2015) but the effect of a varied algal diet on the larval development of *S. kraussii* has not previously been reported. The aim of this study is to assess the effect of different algal diets on the growth, survival rate and mortality of the newly recorded polychaetae, *S. kraussii*, on the rocky shores of Bandar Abbas (Persian Gulf, Iran). This study may be considered as a step for new studies of *S. kraussii* in the Persian Gulf in the future.

MATERIALS AND METHODS

Experimental protocol

All of the algae used in this study were obtained from the Phytoplankton Culture Laboratory, Institution Persian Gulf and Omani Sea of Hormozgan in Iran. Algal species were chosen to cover a wide range of sizes. Four algae with sizes (µm) (mean± SD), *Chlorella vulgaris* (4.72± 0.38), *Nannochloropsis oculata* (3.01± 0.09), *Chaetoceros calcitrans* (10.6± 0.31) and *Tetraselmis chuii* (8.9± 0.34), were used in this study. Reagent grade chemical components of the growth media were obtained from Merck (Germany). All of the algae were cultured in F/2 media (Guillard and Ryther, 1962). The growth chamber was illuminated with cool white fluorescent tubes for 12:12 h light/dark cycles with the intensity ranging from 450 to 540 µmol m^{-1} S^{-1}. Unicellular algae were generally used during their exponential growth phase. The size of the algae and their morphological properties were documented by a Dino-Lite digital microscope camera. The characteristics of these algae were reported (Table 1). *S. kraussii* are found to be particularly amenable to laboratory and experimental studies, being highly abundant in mid-intertidal rocky shores and being very easy to rear in the laboratory on a variety of algal diets. *S. kraussii* were collected from the rocky shores of Bandar Abbas in Iran, returned to the laboratory and induced to spawn. The biology of *S. kraussii* is shown in Table 2. This was accomplished by dividing large clusters of worms into smaller clusters of 2–6 worms, breaking the tubes of these worms and placing a few of these small clusters to gather in finger bowls with filtered seawater for 15 minutes, during which most worms released gametes. For this experiment, gametes from 25 females and 10 males were obtained. The worms were then removed from the finger bowls. The water was swirled for a few seconds to mix the gametes and these were left to fertilize for 10 minutes. The water containing the gametes was passed through a 100 mm screen to filter out large particles, then filtered onto a 25 mm screen to retain the eggs and separate these from the water containing excess sperm, and finally the eggs were gently washed into a 15 beaker filled with seawater. This beaker was placed in a temperature-controlled room at 25°C. The larvae hatched after 38 hours. A large number (about 10,000) of newly

Table 1. Algal genera considered as food for *S. kraussii*

Algal class Species	Properties
Chaetoceros calcitrans	Proven as food
	Have n-3 HuFAs (39%)
	Appropriate size (8-23µ)
Nannochloropsis oculata	Have n-3 HuFAs (39%)
	Small size (3-6µ)
	Proven as food
Tetraselmis chuii	Have n-3 HuFAs (8%)
	Proven as food
	Appropriate size (7-22µ)
Chlorella vulgaris	Without n-HuFAs (0)
	Small size (3-6µ)
	Proven as food

Table 2. Descriptive table of biology of *S. kraussii*

Species	*S. kraussii*
Body size (mm)	7-28
Egg size (mm)	0.070-0.080
Embryonic time (h)	36-48
Larval nutrition	Microalgae
Development time days	15-27
Temperature (°C)	25
Salinity (PSU)	37

hatched trochophores were obtained from this culture, of which 900 larvae were haphazardly collected and transferred into the algal treatments. To count the newly hatched *S. kraussii* larvae, we released the larvae into an 8-liter bucket and swirled the water with two arms until we thought enough agitation was achieved to uniformly distribute the larvae, then we took five 50 ml samples of the water/larvae. Larvae were counted under a stereo microscope (Leica, WILD MZ8) and the number of larvae in the 8-liter bucket was determined. The formula is:

Total number of larvae =
Total number of counting larvae in 5 replicates × 8000 ml water/ 250 ml water

To determine the effect of diet on larval growth, we reared *S. kraussii* larvae in five different microalgae treatments including four single-algal treatments and one multi-algal treatment: (1) *C. calcitrans* (2) *T. chuii* (3) *C. vulgaris* (4) *N. oculata* (5) Multi- algal (Mix of *C. vulgaris*, *N. oculata*, *C. calcitrans* and *T. chuii*). The sizes of algal particles (µm) (mean± SD): C. vulgaris (4.72± 0.38), N. oculata (3.01± 0.09), *C. calcitrans* (10.6± 0.31), *T. chuii* (8.9± 0.34). These are algae present in the ocean at the study site (Persian Gulf, Iran) and therefore naturally available to the *S. kraussii* larvae.

The algal diet experiment was carried out in dishes with aeration at the same temperature (25°C) and salinity (35-37 psu) (salinity in the Persian Gulf). The experimental design consisted of the five algal treatments described above, with five replicates per treatment and 30 larvae per replicate in 10 ml of algal solution. Since the goal of this experiment was to assess the effect of algal type, each algal treatment consisted of high algal densities ($0.5–1\times10^6$ cells/ml) (Pechenik and Tyrell, 2015). For treatments 1 to 4, consisting of a single algal species, a stock suspension of the algae was prepared by diluting concentrated, rapidly growing algal cultures in filtered seawater. The one multi-algal treatment was prepared by mixing equal amounts of the relevant unialgal stock suspensions. Every 3 days, 60% of the water in each dish was replaced with fresh algal suspension, and the water and larvae were transferred to a clean dish. Three days after the start of the feeding trials (i.e. day 5 after fertilization), five larvae were haphazardly removed from each replicate dish, and their body length and width were measured by a Dino-Lite camera at 400×. The same was repeated on days 7, 10 and 15 (N=25 individuals per treatment of 5 replicates per day).

Mortality

The mortality experiment was similar to the experimental design consisting of the five algal treatments described above, except for three replicates per treatment and 10 larvae per replicate. Every 3 days, 60% of the water in each dish was replaced with fresh algal suspension, and the water and larvae were transferred to a clean dish. During each change, the dishes were checked for dead larvae.

Survival Rate

$$SRV\% = ((S - D) / S) \times 100$$

S= Total specimen at each treatment
D= Total mortality

Statistical analyses

To evaluate the effect of the diet treatments on the body length and width of the larvae and mortality, a one-way ANOVA and Tukeý's test for post comparison were performed (Zar, 1984).

RESULTS

Larval growth was significantly affected by algal diet. Larval growth rates were linear in all treatments (*C. vulgaris* R^2 = 0.78; *N. oculata* R^2 = 0.88; *C. calcitrans* R^2 = 0.69; *T. chuii* R^2 = 0.8; mixed algae R^2 = 0.92) (Fig. 1). Larvae in all treatments grew rapidly up to day 5 after fertilization, but after that the larvae growth changed. By day 5, average larval body length differed significantly among treatments ($F= 24.3$, $P<0.05$). Larvae grew significantly faster in 3 treatments (*C. vulgaris*, *N. oculata* and mixed diet) than in *C. calcitrans* and *T. chuii*, and significantly slower in T. *chuii*.

After 15 days of being reared on diets, larval development was greater than on these diets during days 0–10. As expected, after 15 days of development, average larval body length and width differed significantly among treatments (ANOVA, $P<0.05$); those provided with a C. *vulgaris*, *N. oculata* and mixed diet were significantly larger than those that were fed *T. chuii* and *C. calcitrans* as determined by a Tukeý's test. However, algae are not all of equal value to *S. kraussii*. Throughout the study, we did not see dead algae because *S. kraussii* larvae are filter-feeder and thus can feed on dead algae as food. Maximum larval body length was shown in the *N. oculata* diet (Table 3). Between hatching to pre-metamorphosis, larvae were able to capture and ingest particles ranging in size from 3 µm to 11 µm.

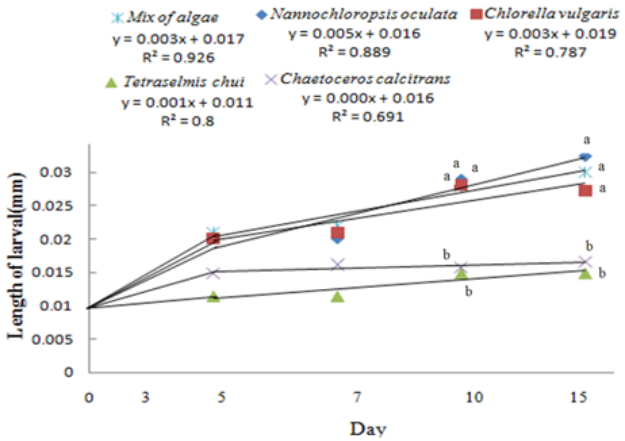

Fig 1. The growth rate of *S. kraussii* larvae in different treatments during 15 days

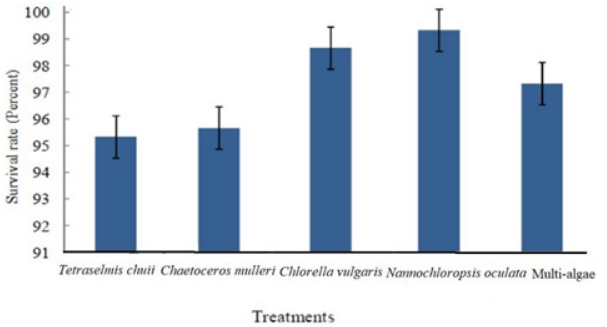

Fig 2. The survival rate of *S. kraussii* in different treatments

Table 3. A maximum larval growth (mm 15 day) according to diets for *S. kraussii* species. Means and standard deviations.

Treatment	Length	Width
Tetraselmis chuii	0.017± 0.002[b]	0.007± 0.00[b]
Nannochloropsis oculata	**0.03± 0.007[a]**	0.014± 0.005[a]
Chaetoceros calcitrans	0.018± 0.001[b]	0.007± 0.0009[b]
Chlorella vulgaris	**0.029± 0.005[a]**	**0.013± 0.004[a]**
Mix of algae	**0.025± 0.004[a]**	**0.012± 0.004[a]**

*Underline represented the highest growth. Different superscripts indicate significant differences between treatments

Mortality and survival rate

Dead larvae were counted regularly to correlate algal feeding with larval density. Mortality was low for larvae (max. 5%); survival rate more than 95%. Fig. 2.

DISCUSSION

When comparing the effects of algae on larval growth, the present results show all tested diets were eaten by the larvae. *C. calcitrans* and *T. chuii* had the largest cell size and were not appreciably consumed by *S. kraussii* larvae in their early stages of development. After hatching (day 1), swimming larvae started to feed but the size of the larvae was small and they had some early cilia thus they were unable to feed on big particles. They were only able to feed on *C. vulgaris* and *N. oculata* during these first days. The swimming larvae ate and grew into larvae with a digestive system. The morphology of the feeding apparatus impacts feeding mechanisms and feeding availability (e.g. phytoplankton species in this study) and different particle sizes affect growth patterns and body size of the larvae (Hansen et al., 1991; Pauly et al., 1985). The physical limitations for food particle ingestion by *S. kraussii* larvae are presumably defined by the dimensions of the oral diameter of the larvae, which limits the size of the phytoplankton that can be consumed. The structure of phytoplankton communities (e.g. phytoplankton size) is extremely important to the feeding function of marine larvae and this function depends on environmental changes which may alter the size structure of the phytoplankton communities (Winder and Sommer, 2012). Some factors such as ingestion rates, digestive rates, assimilation efficiency or differences in nutritional chemistry affect larval growth (Pechenik and Fisher, 1979). The nutritional value of any algal species for a particular organism depends not only on its chemical component, but also on the cell size (Brown and Robert, 2002; Mok et al., 2008). The biochemical component of the algal diet as a food for larvae also influences energy storage (Hansen, 1993). The amount of energy accumulated during the pre-metamorphosis stage influences larval settlement and ability to metamorphose successfully (Jarrett, 2003; Mok et al., 2008). Moreover, for species with feeding larvae, the type of food or food quality has a direct effect on larvae growth and energy reserves (Thiyagarajan and Qian, 2003).

Moreover, *N. oculata* and *C. vulgaris* are without flagella, but *T. chuii* has flagella. Our results suggest that in treatments with *N. oculata*, *C. vulgaris* and mixed algae, there was a rapid larval growth because *N. oculata* and *C. vulgaris* did not have flagella, and were inhabited and did not move but *T. chuii* had flagella and could escape larvae. Also, *C. calcitrans* is a large marine planktonic diatom and it has a chain structure, thus it cannot be consumed as a food by *S. kraussii* larvae at early stages. Thus, *S. kraussii* trochophore larvae at day 7 to day 15 could capture better than trochopore larvae in early stages (despite having a digestive system) of their life cycle; they fed on these algae because of the existing development of their digestive system and growth of their cilia. The digestive system of trochophore larvae in Serpulidae is ciliated. The gut is totally ciliated, the cilia of the oesophagus being arranged in helical rows (Crisp, 1974). Muller-Fegua et al. (2003) showed that the trochophore larvae were able to graze on algae ranging in size from 2 µm to 10 µm. Larval

of polychaetae prefer algae of less than 10µ, the same as mollusk and shrimp larvae (Ryther et al., 1975). Larvae have specific energy requirements through stages of developments and growth. An increase in larval body size tends to lead to an increase of maximum prey and this is perhaps related to the morphological and ontogenetic changes in the ciliary apparatus (Hansen, 1993; Riisgaard et al., 2000). When larvae are larger in late stages, they can capture feed particles via peripheral ciliated band and then transport them to the mouth, often by repeated capture of portions of the band progressively nearer to the mouth (Strathmann, 1987). These properties could be due to the increasing length of the prototroch cilia, the dimensions of the mouth structure, or a contribution from the tentacles (Rassoulzadegan et al., 1984). Thus it suggests that in late stages of larval life, they can feed on big particles. Our results show that phytoplankton species with cells bigger than 7 µm (*T. chuii* and *C. calcitrans*) are commonly consumed by larvae at day 7 or more. When larvae are not suitably fed, they cannot grow. Also, they can be captured by predators as food or not find a suitable place to settle. Thus, with different currents they may be transferred to a different area that does not have a suitable substrate for larvae. This can lead to the offspring of *S. kraussii* becoming extinct in that area. All of the mentioned notes could lead to an underestimate of the abundance of fan worms at the mouth of estuaries (much fine detritus is in suspension there) (Dales, 1957). In the present study, rearing larvae with different algae as food has a different impact on the larval growth and survival. These results suggest that *S. kraussii* larvae have the capacity to feed using alternative sources of energy, and food size and quality can affect their growth and sustainability.

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SAŽETAK

UTJECAJ RAZLIČITIH HRANIDBI BAZIRANIH NA ALGAMA NA STOPU RASTA LIČINKI CRVA CJEVAŠA MNOGOČETINAŠA (SERPULIDAE, POLYCHAETE)

Utjecaj dostupnosti hrane na rast i preživljavanje ličinki *Spirobranchus kraussii* istraživan je hranidbom različitim vrstama algi. Ličinke *S. kraussii* hranjene su s četiri različite vrste morskih mikroalgi, pojedinačno i u različitim smjesama. Najbolji rast zabilježen je pri hranidbi s *C. vulgaris*, *N. oculata*, kao hrana jedne vrste i miješane smjese algi tijekom 15. dana nakon oplodnje. Smrtnost ličinki je bila niska (maks. 5%); a preživljavanje više od 95%. Ovi rezultati ukazuju na to da ličinke *S. kraussii* imaju sposobnost hraniti se alternativnim izvorima energije, a veličina i kvaliteta hrane mogu utjecati na njihov rast i održivost.

Ključne riječi: Ishrana, rast, Serpulidae, stopa preživljavanja, smrtnost, Perzijski zaljev

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