HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 45, 345–356 (1991)

Effects of seven diets on the population dynamics of laboratory cultured *Tisbe holothuriae* Humes (Copepoda, Harpacticoida)

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ABSTRACT: The harpacticoid copepod *Tisbe holothuriae* was collected from Saronicos Gulf (Greece) and reared under constant laboratory conditions. In order to study the effects of food on the population dynamics, seven diets were tested: the seaweed *Ulva*; five artificial compound feeds: the liquid Fryfood® (Waterlife), a powder of *Mytilus*, yeast, soya and *Spirulina*, respectively; and a mixed diet consisting of *Ulva* and Fryfood. The life cycle parameters (mortality, sex ratio, generation time, offspring production) were measured, and the demographic variables [mean generation time (T), net reproductive rate (Ro), and intrinsic rate of natural increase (r_m)] were determined. As to their efficiency regarding population dynamics, the diets ranked as follows: (1) *Ulva* + Fryfood, (2), *Ulva*, (3) Fryfood, (4) *Mytilus*, (5) soya, (6) yeast, and (7) *Spirulina*. In this order they cause a progressive increase of both larval mortality and generation time, a progressive decrease of sex ratio, number of offspring per egg sac, number of egg sacs per female and, consequently, of Ro and r_m. The observed differences between diets were most pronounced with respect to offspring production. Of the compound diets, those containing animal extracts were more efficient than those containing vegetable materials. *Ulva* plays an important role in the nutrition of *T. holothuriae*, favouring offspring production as well as larval survival, development and pigmentation. *Ulva* in combination with Fryfood led to a greater copepodid survival and offspring production. This mixed diet proved to be the most favourable for rearing the Greek population of *T. holothuriae*, resulting in an efficient intrinsic rate of natural increase (r_m = 0.304) of the population.

INTRODUCTION

Mixed diets including *Artemia*, rotifers, copepods and other microfauna species have been proved to give the best growth of fish larvae (Nash & Kuo, 1975; Kuhlman et al., 1981). Of the copepods, harpacticoid copepods are the most suitable live food organisms (Rothbard, 1976; Fujita, 1977; Gopalan, 1977; Nash, 1977; Kahan, 1981).

The harpacticoid copepod *Tisbe* is referred to in the literature as an appropriate live prey for fish larvae in aquaculture (Uhlig, 1980; Kahan et al., 1982). In addition, *Tisbe* is a very suitable test organism in environmental physiology and toxicology. Furthermore, the detailed determination of the various life cycle parameters of *Tisbe holothuriae* living in Greek sea waters could provide useful data for taxonomists: the systematics of the genus *Tisbe* is complicated because of the presence of morphologically very similar species or sibling species. According to Battaglia & Volkmann-Rocco (1973), differences in the biological cycle would support taxonomical separation.

At the Zoological Laboratory of the University of Athens we have undertaken a series of experiments on the importance of abiotic and biotic factors for the population dynamics of the harpacticoid copepod *T. holothuriae* Humes. These experiments, supported by the Greek Ministry of Agriculture, aim to study the possibility of using *Tisbe* living in Greek sea waters as food for fish larvae in aquaculture. This paper describes the effects of diet on the various life cycle parameters of a Greek population of *T. holothuriae.*

MATERIAL AND METHODS

Tisbe holothuriae Humes is a marine epibenthic copepod with widespread distribution in the coastal environment. The animals used in these experiments were collected from the Saronicos Gulf of Greece (Lagonissi area) and cultured under laboratory conditions. In order to allow acclimatization, work began on the second generation derived from the wild population. Preliminary experiments showed increased mortality and lower reproductive capacity of the first generation. Offspring up to the fourth generation only were used in order to avoid inbreeding effects.

All experiments were run in constant-temperature rooms at 19 °C (± 0.5) and 38 ‰ S. These conditions were proved, in previous experiments (Miliou & Moraitou-Apostolopoulou, in press), to be the most favourable for rearing the Greek population of *T. holothuriae.* A number of fertilized females G1 (second generation derived from the wild generation) were isolated from the mass culture (a single mating is sufficient to ensure production of several egg sacs by a female). Their offspring were observed daily until the appearance of fertilized females G2. When the G2 females were carrying their first egg sac, they were put individually into 50-ml bowls. Ten to twenty nine females were used for each experiment. After the appearance of a new egg sac, the females were transferred to a new bowl. The larvae from the previous egg sac were left in the original container and gave the G3.

All bowls were examined daily and the following parameters of the biological cycle were measured:

(1) Times from hatching of G2 females to the release of their sucessive broods (generation times, x). The minimum generation time is the age of G2 females at the release of nauplii from the first egg sac.

(2) Number of nauplii at the time of hatching (no. of offspring per egg sac).

(3) Number of egg sacs per female.

(4) Total number of offspring per female.

(5) Mortality during development. The G3 offspring were observed daily for 12 days. Nauplii and copepodids were counted under a binocular microscope and transferred to new containers. For each trophic condition, 15 recipients were used with about 40 newly hatched nauplii each. The LT_{50} was calculated from the daily percentage mortality: time needed for the death of 50 % of the individuals (UNEP: Reference methods for marine pollution studies, 1987).

(6) Sex ratio. The percentage of females among G3 adults.

(7) Number of G3 females derived from G2 females.

The measurement of these parameters enables the determination of the demographic variables: mean generation time (T), net reproductive rate (Ro), and intrinsic rate of natural increase (r_m), which allow a prediction as to the capacity of *T. holothuriae* populations to proliferate under given environmental conditions.

The mean generation time (T) is defined as $T = \frac{\Sigma(x \cdot Ux)}{\Sigma Ux}$, where x is the age (in days) of females G2 at the time of hatching of nauplii and Ux is the number of nauplii per egg sac. The net reproductive rate $Ro = \frac{G3}{G2}$ indicates the replacement rate of ovigerous females G2 by their female progeny G3 from one generation to another. The intrinsic rate of natural increase (r_m) results from the relation $r_m = \frac{\ln Ro}{T}$.

General methods for the calculation of the demographic variables are given by Andrewartha & Birch (1954). The method applied to *Tisbe* population in this work is that of Gaudy & Guérin (1977).

Seven different diets were tested: (1) *Ulva* + Fryfood; (2) *Ulva*; (3) Fryfood; (4) *Mytilus*; (5) soya; (6) yeast; and (7) *Spirulina*.

Ulva was collected from the same biotope in which *Tisbe* was found. The fronds of *Ulva*, cleaned of microorganisms and cysts under a binocular microscope, were cut into small pieces.

Mytilus soft parts were dried at 60 °C for 24 h, finely ground and kept at 4 °C.

Fryfood® (Waterlife) is a liquid product of high nutritional value. It is composed of the following micronised ingredients: *Artemia, Daphnia, Spirulina, Mysis*, yeast, whitefish, whole egg, mosquito larvae, spinach, and is enriched with the vitamins A, B1, B2, B6, B12, C, E.

Soya granular. It consists of 41.4 % proteins, 2 % phospholipids, 19 % fatty acids, 25 % carbohydrates, 0.2 % calcium, 0.6 % phosphates and a variety of enzymes.

Yeast superzym. Rich in amino-acids, metals, enzymes and a rich vitamin complex.

Spirulina (Lanes). It is composed of the phytoplanktonic organism *Spirulina*. It is the richest of all tested diets in proteins (68 %), and contains 21 amino-acids including phenylalanine.

The selection of these types of food was based on the literature and their local availability. The aim of the experiments was to investigate the qualitative rather than the quantitative effects of food. Therefore, food was offered in excess, its quantity being adjusted to avoid accumulation of discarded material between feeding times.

Nauplii and older stages fed actively on food particles. Fryfood, a dense non-miscible liquid product, accumulated at the bottom of the containers. Copepods came into contact and fed directly on it. According to Gillet & Guérin (1976) and Uhlig (1980), the food particles offered to *Tisbe* must not exceed 250 µm in diameter. Larger particles were removed using a plankton net of an appropriate mesh size.

For the statistical evaluation, the pairs (Mann-Whitney) nonparametric test was applied as the size of the samples was smaller than 40. Using normal approximation, we calculated the test statistic z. Since $z > 1.96$ we reject the null hypothesis that the samples have the same median. In the results, the two tailed probability (P) of equaling or exceeding z is given. If $P \leq 0.05$, the samples have a statistically significant difference (Zar, 1984).

RESULTS

Table 1 shows the daily percentage mortality of the G3 offspring for each tested diet. The mortality curves were transformed to linear lines according to the equation:

$y = a + b\ln x$; y: percentage mortality, x: day.

Table 1. Daily percentage mortality of *Tisbe holothuriae* during larval development under different food conditions

Day	*Ulva* + Fryfood	*Ulva*	Fryfood	*Mytilus*	Soya	Yeast	*Spirulina*
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	12.12	13.43	15.63	17.07	17.86	19.57	21.95
3	19.70	20.14	23.13	25.61	26.98	27.83	36.59
4	24.24	25.69	28.13	29.88	30.95	33.04	41.87
5	28.41	30.79	33.75	35.37	36.90	39.13	48.78
6	31.82	34.95	37.50	39.63	40.87	45.65	53.66
7	34.47	38.66	41.25	43.60	47.62	53.91	62.60
8	36.74	43.75	48.13	50.91	52.78	60.87	67.89
9	38.64	49.31	53.13	57.01	61.11	67.83	73.17
10	40.53	54.17	58.13	60.98	63.89	70.87	79.67
11	42.42	59.72	63.75	64.94	69.05	76.09	82.93
12	43.94	62.73	66.88	68.90	73.02	80.87	85.77

This linear regression line proved to be the "best fit" line through the data of the percentage mortality per day. The parameters a and b were calculated using the criterion of the least squares. The obtained values of the constant a, the regression coefficient b and the correlation coefficient r, for each type of food, are given in Table 2.

This smoothing extrapolation technique allows the calculation of the time (LT_{50}) needed for 50 % death of the initial nauplii. Figure 1a shows the calculated values of LT_{50} for the seven diets. It can be seen that the mixed diet consisting of *Ulva* and Fryfood is the most favourable for the survival of *Tisbe*, while *Spirulina* is the least favourable.

Figure 1b presents the sex ratio of *Tisbe* fed on the different diets. As in the case of survival, the mixture of *Ulva* and Fryfood proved to be the most favourable, giving the highest percentage of females, while *Spirulina* proved to be the least favourable.

Table 3 shows the mean values (and standard deviations) of some main life cycle parameters (generation times, no offspring of G2 females) for each type of food. Figures 1c–f give the mean values of: minimum generation time (Fig. 1c); number of the first egg sac offspring (Fig. 1d); number of egg sacs per female (Fig. 1e); total number of offspring

Table 2. Constant a, regression coefficient b, correlation coefficient r, and standard error of estimate (S.E.E.) of the linear regression for daily percentage mortality (Table 1) under different food conditions

Type of food	a	b	r	S.E.E.
Ulva + Fryfood	0.008	17.657	0.0098	0.1693
Ulva	–10.914	27.688	0.9763	3.6953
Fryfood	–9.659	28.799	0.9752	3.9357
Mytilus	–8.051	29.157	0.9773	3.8004
Soya	–9.327	31.201	0.9763	4.1575
Yeast	–12.277	35.558	0.9784	4.5196
Spirulina	–5.930	36.027	0.9908	2.9518

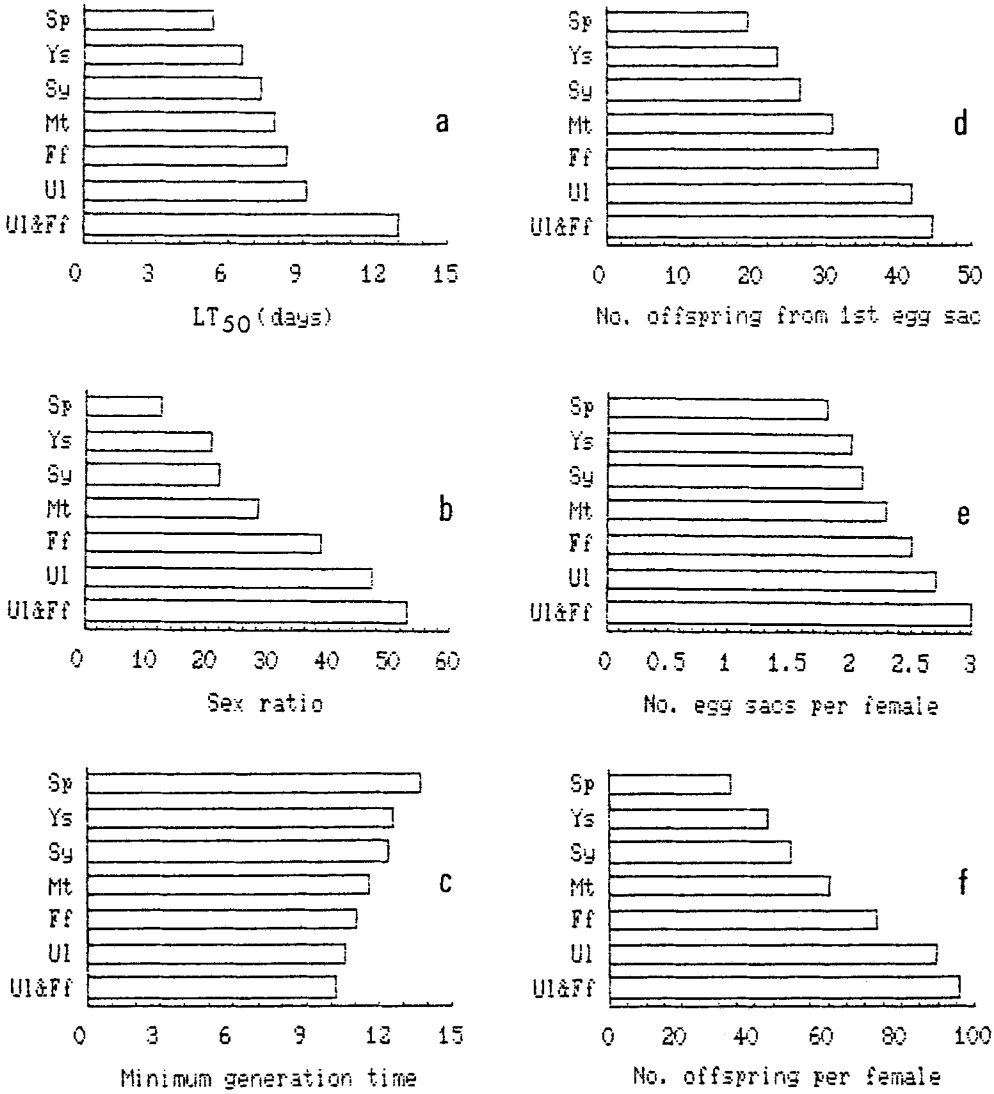

Fig. 1. Histograms illustrating the effects of seven diets on (a): LT_{50}, (b): sex ratio (% females among adults), (c): minimum generation time, (d): no. offspring from the first egg sac, (e): no. egg sacs per female, (f): no. offspring per female

per female (Fig. 1f). It is important to note that the first egg sac always gave a greater number of offspring than the subsequent egg sacs, under all trophic conditions. It is for this reason that parameter (d) is taken into account here.

From the above measurements we calculated the three demographic variables: T (mean generation time), Ro (net reproductive rate) and r_m (intrinsic rate of natural increase). The obtained values are illustrated in Figures 2a, 2b, and 2c, respectively. For these calculations, only the generation times (x) and the number of offspring (Ux) of the

Table 3. *Tisbe holothuriae:* mean values ± SD of some main life cycle parameters related to different diets

Diet	Generation time (1st egg sac)	Generation time (2nd egg sac)	No. offspring from 1st egg sac	No. offspring from 2nd egg sac	No. offspring from 3rd egg sac	No. G3♀♀ / No. G2♀♀
Ulva + Fryfood	10.2 ± 0.40	12.7 ± 0.87	44.6 ± 5.12	25.7 ± 2.68	17.6 ± 1.25	468/16
Ulva	10.6 ± 0.49	13.1 ± 0.70	41.6 ± 4.53	23.4 ± 4.76	15.6 ± 4.96	210/15
Fryfood	11.1 ± 0.83	13.8 ± 0.74	37.1 ± 4.74	21.7 ± 4.60	14.5 ± 1.50	102/12
Mytilus	11.6 ± 1.20	14.2 ± 1.25	31.0 ± 3.83	19.2 ± 5.07	10.5 ± 1.50	66/15
Soya	12.4 ± 1.28	14.9 ± 1.13	26.5 ± 4.68	15.0 ± 3.39	8.5 ± 1.50	39/15
Yeast	12.6 ± 1.20	15.5 ± 1.20	23.5 ± 3.59	13.3 ± 2.87	7.0 ± 0.00	21/15
Spirulina	13.7 ± 1.61	15.9 ± 1.04	19.5 ± 3.59	11.0 ± 2.00	3.0 ± 0.00	6/12

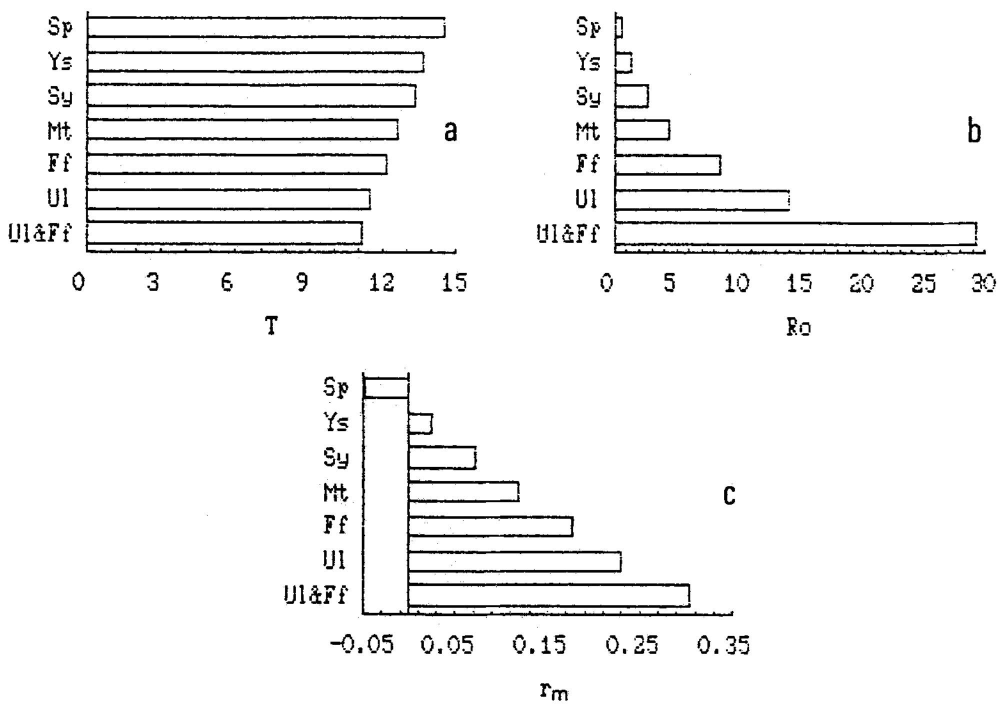

Fig. 2. Histograms illustrating the effects of seven diets on the demographic variables of the population dynamics of *Tisbe holothuriae*. a: mean generation time (T), b: net reproductive rate (Ro), c: intrinsic rate of natural increase (r_m)

first two egg sacs are taken into account, as these gave the majority of a female's total offspring (see Table 3).

The histograms (Figs 1 and 2) reveal the following rank of the diets as far as their efficiency regarding *Tisbe* productivity is concerned: (1) *Ulva* + Fryfood, (2) *Ulva*, (3) Fryfood, (4) *Mytilus*, (5) soya, (6) yeast, and (7) *Spirulina*.

In this order, the diets cause a progressive decrease of LT_{50} (Fig. 1a), sex ratio (Fig. 1b), number of offspring from the first egg sac (Fig. 1d), number of egg sacs per female (Fig. 1e), total number of eggs (Fig. 1f), and Ro (net reproductive rate), which depends primarily on offspring production, survival and sex ratio (Fig. 2b).

In contrast, in the same order the diets give a progressive increase of the time needed for the appearance of the nauplii of the first egg sac (Fig. 1c), causing an increase of T (Fig. 2a) which depends mainly on the time needed for development and maturation.

In the same order, the r_m-values (Fig. 2c) show a progressive reduction, turning negative for *Spirulina*. This means that *Spirulina* leads to decline and finally disappearance of the initial population.

The Mann-Whitney test has been applied to each pair of values of (a) the percentage mortality in respective days, (b) the minimum generation time, and (c) the offspring production from the first egg sac. Table 4 shows the two tailed probability (P) of equaling

Table 4. Mann-Whitney nonparametric test: two tailed probability (P) of equaling or exceeding z. $P \leq 0.05$: significant differences

			Mortality during development				
	Ulva + Fryfood	*Ulva*	Fryfood	*Mytilus*	Soya	Yeast	*Spirulina*
Ulva + Fryfood	*						
Ulva	0.2643	*					
Fryfood	0.1679	0.6936	*				
Mytilus	0.0878	0.4307	0.7427	*			
Soya	0.0569	0.2934	0.5994	0.6458	*		
Yeast	0.0302	0.1486	0.2643	0.3933	0.5545	*	
Spirulina	0.0039	0.0356	0.0569	0.1007	0.1486	0.3933	*
			Minimum generation time				
	Ulva + Fryfood	*Ulva*	Fryfood	*Mytilus*	Soya	Yeast	*Spirulina*
Ulva + Fryfood	*						
Ulva	0.2039	*					
Fryfood	0.0357	0.1787	*				
Mytilus	0.0099	0.0484	0.4298	*			
Soya	0.0004	0.0012	0.0341	0.2129	*		
Yeast	0.0003	0.0006	0.0111	0.1033	0.6970	*	
Spirulina	0.0001	0.0001	0.0007	0.0078	0.0807	0.1603	*
			No. offspring from 1st egg sac				
	Ulva + Fryfood	*Ulva*	Fryfood	*Mytilus*	Soya	Yeast	*Spirulina*
Ulva + Fryfood	*						
Ulva	0.4691	*					
Fryfood	0.0093	0.1994	*				
Mytilus	0.0051	0.0103	0.0776	*			
Soya	0.0051	0.0051	0.0202	0.1720	*		
Yeast	0.0051	0.0051	0.0082	0.0247	0.2963	*	
Spirulina	0.0051	0.0051	0.0051	0.0064	0.0542	0.1269	*

or exceeding z. The statistical treatment of the three parameters mentioned above reveals the existence of subgroups in this classification of types of food, depending on the tested parameter.

Concerning mortality, the mixed diet of *Ulva* + Fryfood is different from the other foods (only from yeast and *Spirulina* in statistically significant levels), causing an important decrease of mortality. Concerning minimum generation time, the diets *Ulva* + Fryfood and *Ulva* alone are clearly separated from the others. *Ulva* causes an important decrease of the time needed for development and maturation of offspring.

As to offspring production, the mixed diet *Ulva* + Fryfood, and *Ulva* and Fryfood given separately, show similar results, significantly different from the other diets. The number of offspring from the first egg sac increases with both Fryfood and *Ulva*, but especially when these are offered simultaneously.

With respect to all parameters considered, *Spirulina* was the least efficient food. *Mytilus* and Fryfood gave almost the same results, and this was also the case for soya and yeast.

The differences between the seven diets as to offspring production and generation time were more pronounced than those concerning mortality which were often not statistically significant.

DISCUSSION

Tisbe is known to demonstrate an opportunistic feeding behaviour, utilizing various sources of food such as bacteria, unicellular algae, detritus, vegetables (Battaglia, 1970; Gaudy & Guérin, 1977; Rieper, 1978). However, the study of the influence of diet on the population dynamics of *Tisbe* has proved that food is an important factor in the regulation of the productivity of *Tisbe* populations. All life cycle parameters were affected to a greater or lesser degree by the type of food offered. Gaudy & Guérin (1977), feeding *Tisbe* with the two diets Germalyne and Renutryl, found also significant variations in population measurements dependent on diet.

The demographic variable r_m (intrinsic rate of natural increase) which characterizes population proliferation, was found to vary from 0.304 under the best nutritional conditions (*Ulva* + Fryfood) to 0.24 for yeast. For *Spirulina*, negative values of r_m (-0.048) have been noted. The r_m values obtained under optimal trophic conditions for the Greek population of *Tisbe holothuriae* are considered satisfactory, compared with the maximum r_m: 0.301 (Aliment Bioter) and 0.313 (Renutryl), found by Gaudy & Guérin (1977) and Gaudy et al. (1982), respectively, for the French population of *Tisbe holothuriae*, under the same temperature/salinity conditions.

The productivity of *Tisbe* populations is directly correlated with the following life cycle parameters: (a) survival during development, (b) sex ratio, (c) number of egg sacs per female, (d) number of offspring per egg sac, (e) development rate. One type of food might favourably affect one particular parameter, e.g. *Ulva* substantially increases the developmental rate of nauplii and copepodids, decreasing the mortality especially of nauplii. On the other hand, Fryfood, in comparison with the other compound diets, causes an important increase of offspring production and lowers the mortality especially of copepodids. Of the seven tested diets, the mixed diet of *Ulva* + Fryfood gives the best results. Either component has a substantially favourable effect on different parameters of the population dynamics.

The effectiveness of a type of food depends on its digestibility and on how it fulfils the nutritional requirements of the species. The food quality has been proved to be particularly important in crustaceans especially for fertilitiy and fecundity (Provasoli et al., 1959).

The amino-acid content does not seem to be a decisive factor in the effectiveness of a food, since *Spirulina*, although richest in amino-acid, was the least efficient one. The amino-acid composition of copepods is of a somewhat conservative nature (Raymont et al., 1975). The fatty-acid content of food seems to influence copepod productivity, especially survival of copepodids and number of eggs.

Studies on the chemical composition of copepods have shown that their fatty acids are dominated by (n-3) polyunsaturated fatty acids (P.U.F.A.) (Sargent & Falk-Petersen, 1981). It is noteworthy that in the presence of a substantial input of dietary fatty-acids, especially polyunsaturated ones, the de novo fatty-acid biosynthesis no longer proceeds. The conversion of dietary wax esters into fatty acids may be advantageous to the animal as this requires little or no energy expenditure (Wakil et al., 1983). *Mytilus* is a food rich in lipids, particularly in unsaturated fatty acids. Fryfood is also enriched with unsaturated fatty acids. In contrast, *Spirulina* with a low fatty acid content proved to be inefficient. Soya is more efficient than yeast, and is also richer in lipids.

In the present study, the pure animal feed (*Mytilus*) and animal-derived feeds (Fryfood) proved to be more efficient than the compound vegetarian feeds (soya, yeast, *Spirulina*). Guérin & Gaudy (1977) suggest that *Tisbe holothuriae* displays a higher productivity and lipid content when fed on compound artificial diets of animal substances than when fed on diets of vegetarian origin or on living unicellular algae. Gopalan (1977) also mentioned that the cultures of the harpacticoid copepod *Nitocra spinipes* Boek displayed higher productivity when fed on shrimp head meal than on live *Chlorella*.

Diet-induced differences in enzymatic processes of copepods seem to play an important role in the effectiveness of a food. In *Tisbe*, varying trophic conditions are responsible for differences in the relative activity of different isoenzymes (Guérin & Kerabrun, 1982). Hirche (1981) found that the amylase activity in adult females positively correlated with the chlorophyll a concentration of the diet. This observation can further explain the efficiency of *Ulva*. Furthermore, the activity of a basic digestive enzyme of zooplankton (laminarase) was found to be more intense when food is rich in laminarin, a substance abundant in marine plants (Hasset & Landry, 1982).

In our experiments, a total loss of pigmentation was observed when *Tisbe* was fed on any one food exclusively, with the exception, however, of *Ulva*. Tanaka et al. (1976) reported that the total carotenoid content in crustaceans varies according to food type.

Mixed diets seem to fulfil the nutritional requirements for high *Tisbe* productivity better than other diets, probably due to a richer supply of trace elements and vitamins. Fryfood, which proved to be a very efficient feed ($r_m = 0.177$), contains a variety of items (plants and animals). Furthermore, Fryfood is enriched with vitamins and trace metals. Its combination with the live alga *Ulva* can also be considered a highly suitable diet for *Tisbe*. Takano (1971) reported that when a combination of live algae with non-living materials was fed to *Tigriopus japonicus*, the population density of the latter increased faster for 2 to 6 days than was the case using separated foods.

The favourable influence of a type of food may not be directly related to its chemical composition. For example, the fronds of *Ulva* significantly increase the substratum available to *Tisbe*. Gaudy & Guérin (1979) report that the increase in the surface-volume ratio in breeding tanks results in an increase in *Tisbe* production. Moreover, in the presence of a live seaweed, oxygen is supplied and toxic compounds of nitrogen and other elements are absorbed through the process of photosynthesis (Harlin, 1978). Finally, the efficiency of the fronds as shelter should not be overlooked (Itami & Yoshinori, 1977).

Fryfood, apart from the variety of food items, offers a large particle spectrum that corresponds to the changing size of the *Tisbe* oral parts in the course of development. The substantial decrease in copepodid mortality and the increase in offspring production when *Tisbe* is fed on Fryfood must be attributed to this fact as well. Nassogne (1970) reports that *Euterpina acutifrons* has a higher growth rate and reproductive capacity when fed on a mixture of materials of different sizes, as nauplii cannot eat particles larger than 16 μm, and adults cannot ingest particles smaller than 7 μm.

Seaweeds (Rothbard, 1976) and vegetables (Kahan, 1979) become available as food to nauplii and copepodids when the latter perforate the small cells of the epidermal cuticle of these plants. This fact could contribute to the low mortality of the nauplii of *Tisbe* fed on *Ulva*.

Finally, the success of a diet is also related to its capability of offering an appropriate substratum for bacteria to proliferate (Coull, 1973; Rieper, 1978). In benthic harpacticoid copepods, the rate of bacteria assimilation is 8 to 10 times higher than in phytoplankton (Brown & Sibert, 1977). In addition, bacteria serve as food to microzooplankton (mainly ciliates) on which the harpacticoid copepods feed (Rieper & Flotow, 1981). *Ulva* becomes a food source both directly and indirectly, acting as substratum for adhesive diatoms, bacteria and organic particle accumulation (Rothbard, 1976).

The mixture *Ulva* + Fryfood offers all these advantages and proved to be the most satisfactory diet for *Tisbe* production ($r_m = 0.304$).

Gaudy & Guérin (1979) suggested that a perfect diet, besides its being suitable for optimum production, should be cheap and simple. The liquid Fryfood offers simplicity in the maintenance of the cultures as it represents an easily available, qualitatively adapted food supply. The extensive natural quantities of *Ulva* provide a low-cost and very accessible supply of this food resource. The mixed diet of *Ulva* and Fryfood fulfils all the above mentioned criteria, and so we suggest that it is suitable for mass production of *Tisbe*.

Acknowledgements. We wish to thank Dr. G. Verriopoulos for continuous support throughout this study and his valuable advice. This research was financially supported by the Greek Ministry of Agriculture (Department of Fisheries and Aquaculture).

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