

FEEDING BEHAVIOR OF *Apocyclops spartinus* (COPEPODA: CYCLOPOIDA) ON *Pavlova lutheri* (HAPTOPHYTA) IN LABORATORY CONDITIONS

COMPORTAMIENTO ALIMENTARIO DE *Apocyclops spartinus* (COPEPODA: CYCLOPOIDA) SOBRE *Pavlova lutheri* (HAPTOPHYTA) EN CONDICIONES DE LABORATORIO

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Abstract

The copepod *Apocyclops spartinus*'s predatory behavior was described on naupliar and adult stages (males and females) in function of its mandibular border under laboratory conditions. To describe this, filtration and ingestion rates on microalgae *Pavlova lutheri* were estimated in three copepod ontogenetic phases: 1) nauplii (i-iii), 2) metanauplii (iv-vi) and 3) adults (males and females) between 3 to 30 hours of consumption in five intervals, at 24 °C and 35 PSU (Practical Salinity Units). Ingestion rate was higher in females (60.7%) than in males (36.1%), metanauplii (2.5%) and nauplii (0.6%). Highest filtration rate was recorded by the metanauplii (52.3%), followed by the females (24.2%), males (12%) and nauplii (11.5%). The potential and exponential models described the relationship between ingestion/hours and filtration/hours, those recorded parameters as negative superindexes, explaining higher dietary demand by adults over naupliar stages and depicted an asymptote for established balance between copepod satiety and food rejection due to the loss of nutritional value. In summary, Itoh's mandibular edge index classified *A. spartinus* within the carnivorous spectrum; however, its feeding behavior, which relies on mobile microalgae, demonstrated an active ambush predatory strategy rather than passive foraging.

Key words: culture, clearance rate, ingestion rate, mandible edge index, mathematical models.

Resumen

Se describió el comportamiento predatorio del copépodo *Apocyclops spartinus* en función de su borde mandibular en condiciones de laboratorio. Para describirlo, se estimaron las tasas de filtración e ingestión con la microalga *Pavlova lutheri* en tres fases ontogénicas del copépodo: 1) nauplios (i-iii), 2) metanauplios (iv-vi) y 3) adultos (machos y hembras) entre 3 y 30 horas de consumo en cinco intervalos, a 24 °C y 35 UPS (Unidades Prácticas de Salinidad). La tasa de ingestión fue mayor en las hembras (60.7%) que en los machos (36.1%), seguido por metanauplios (2.5%) y nauplios (0.6%). La mayor tasa de filtración la registraron los metanauplios (52.3%), seguidos de las hembras (24.2%), los machos (12%) y los nauplios (11.5%). La relación entre ingestión/horas y filtración/horas fue registrado con los modelos potencial y exponencial, cuyos parámetros fueron superíndices negativos, explicando una mayor demanda de alimento por parte de los adultos sobre los estadios naupliar y representando una asíntota para el equilibrio establecido entre la saciedad del copépodo y el rechazo del alimento debido a la pérdida del valor nutritivo. En síntesis, el índice de borde mandibular de Itoh clasificó a *A. spartinus* dentro del espectro carnívoro; sin embargo, su comportamiento alimentario, basado en microalgas móviles, demostró una estrategia depredadora de emboscada activa más que de forrajeo pasivo.

Palabras clave: cultivo de copépodos, tasa de aclaramiento, tasa de ingestión, índice de borde mandibular, modelos matemáticos.

Introduction

One way to transfer energy between living organisms is food assimilation, so its quantification is a key factor of trophic interactions studies. Zooplankton grazing on autotrophic organisms is considered a process that explains the transfer of energy from the first trophic level to higher levels due the consumption of primary production (Conover & Huntley, 1980; Ramlee *et al.*, 2021). Mauchline *et al.* (1998) point out that the grazing pressure of herbivorous copepods on phytoplankton is based on a growth and metabolism function, so due to the abundance of copepods, it assumes their relevant role in aquatic ecosystems.

Although, the term herbivorous have been used to explain the pressure of copepods over microalgae without considering its motile or non-motile feature. The magnitude of these roving characteristics determines feeding behavior of copepods, and it may diversify their conducts towards herbivory or omnivory (Chen *et al.*, 2018), even predatory (Saiz & Kiørboe, 1995).

Copepods feeding behavior can be described from the morphological study of their mandibles (Itoh, 1970; Sługocki, 2020). The dental structures derived from the mandibular bases (gnathobases) serve as specialized mechanical apparatus for feeding. These structures are

characterized by architecture, composition and chemical properties, which enable them to effectively shred phytoplankton (Michels & Schnack-Schiel, 2005; Harvey & Butterfield, 2022). These mandibular teeth are shaped like fangs and have a silica covering on the chitinous skeleton base, evidencing the co-evolution between the predator and its prey (Michels & Gorb, 2015). In addition, this characteristic explains another reason why copepods are dominant and adaptable to different environments (Chen *et al.*, 2018). Consumption strategies, prey preferences, and food demand in copepods are intricately linked to species-specific characteristics, as well as variations related to stages and sex (Allan *et al.*, 1977; Im *et al.*, 2015). Thus, becomes meaningful to integrate morphology with feeding behaviors, especially in species with significant economic potential. Understanding these relationships not only enhances our comprehension of copepod ecology but also provides sustainable management practices for business activities.

One of the main goals for quantifying feeding parameters in zooplankton is to find reliable and convergent sources to match the formula that corresponds to their respective dimensionless analysis (Harvey, 1937; Betouhim-El & Kahan, 1972; Frost, 1972; Abu-Rezq *et al.*, 1997) and thus avoid such omissions (Brito *et al.* 2006). The other point is to express the significance of each value obtained within a trend explained in non-linear mathematical models, a method as much useful as friendly, becoming necessary to simulate the dynamics of these processes and develop replicable strategies in larger scale systems (Tedeschi, 2006), in this case for decision making in productive or experimental aquaculture applications. However, polynomial models have been used in *Euterpina acutifrons* to explain its feeding behavior (Sautour & Castel, 1993) but the formula replication was not clear also the interpretation of these parameters.

Since the modeling of ingestion and filtration rates allows the calculation of the required or expected feed (Mullin *et al.*, 1975; Larsen & Riisgård, 2022), the corresponding quantification tools are the filtration rates or water clearance, the ingestion rate or daily ration and the consumption coefficient also known as grazing rate (Båmstedt *et al.*, 2000). Mauchline *et al.* (1998) observed that some current models describe the process of feeding copepods in a mechanistic way and tend to consider filtering organisms as passive filter feeder with intake predictions based on microalgae concentration and size. They argue that feeding depends on the complex behavior of each species and propose to evaluate new models to obtain realistic predictions according to the expected differences between culture conditions and the natural environment.

The genus *Apocyclops* has been reported for commercial use in aquaculture in the USA (Phelps *et*

al. 2005), Nicaragua (Cano *et al.*, 2004), Iran (Farhadian *et al.*, 2008) and Taiwan (Jepsen *et al.*, 2021). However, few essays have been conducted on *Apocyclops spartinus* since its description by Ruber (1968) and Bircher's toxicological study (Bircher & Ruber, 1988), so it is valuable to know the feeding behavior for a poorly documented taxon. Previous observations in our laboratory allowed us to determine that cultures of *A. spartinus* were normally developed with *Pavlova lutheri* (Haptophyta), *Tetraselmis* spp. (Chlorophyta) and diatoms such as *Skeletonema* spp. and *Chaetoceros* spp. (Bacillariophyta), but their population growth decreased when fed *Nannochloris* spp. or *Nannochloropsis* spp. (Ochromphyta), these latter being characterized by little movement (Appendix 1). Hence, *P. lutheri* was selected due to its small size, fast movements and recognized aquaculture value (Beaumont *et al.*, 2004), its potential for cultivation, attributed to its elevated levels of neutral lipids and glycolipids, as well as, the presence of Eicosapentaenoic acid and Docosahexaenoic acid (Meireles *et al.*, 2003; Ganga *et al.*, 2021). Furthermore, *P. lutheri* is a marine microalgae noted for its ability to produce significant amounts of phytosterols, establishing it as an important model for investigating lipid biosynthesis in eukaryotic algae (Ahmed *et al.*, 2015).

This article hypothesized that the feeding strategy of *Apocyclops spartinus* has a predatory carnivorous character, based on the morphology of its mandibular edge. Besides it is suggested two non-linear models trends to represent the expected amount of food required within a thirty-hour interval.

Materials and methods

Biological material

The copepod *Apocyclops spartinus* (Copepoda: Cyclopoida) and the microalgae *Pavlova lutheri* (Haptophyta) were cultured in the "Banco de Germoplasma de Organismos Acuáticos" (BGOA) of the Instituto del Mar del Perú (IMARPE).

A. spartinus was collected from the wetlands of Ventanilla, Callao, Peru (11°52'16.0 "S; 77°08'19.2 "W) in 2009. The copepods were isolated through successive pipetting and washing between droplets of filtered and sterile seawater on a glass slide, similar to the methodology outlined by Andersen (2005). Then they were coded as the strain IMP-BG-Z014. Meanwhile, *P. lutheri* was purchased from the University of Texas at Austin, coded as UTEX LB 1293. These organisms were maintained inside a Torrey R-14AI climatic chamber under controlled conditions of temperature (24 ± 1 °C), illumination (60 µM s-1m-2), 14:10 h photoperiod (light: dark) and salinity of 35 PSU without aeration. Seawater was filtered at 0.22 µm and sterilised gradually to reach 105 °C for 10 min. Specific morphological identification was based on the work of Coelho-Botelho (1999) and

Elías-Gutiérrez *et al.* (2008) with optical and scanning electron microscopy images.

From each conditioned monoculture, two groups of 30 gravid females were carefully selected and placed in two 100 ml beakers containing seawater enriched with *P. lutheri* at a concentration of 10^3 cells/ml. Following a 24-hour incubation period, the adult females were removed to facilitate the collection and observation of the nauplii for subsequent experimentation.

The determination of naupliar sub-stages was conducted in accordance with the criteria established by Czaika (1982) and the pictorial key provided by Miracle (2015). The naupliar period comprises three distinct stages (N1 to N3), each lasting less than two days at a controlled temperature of 24 °C. Morphologically, these stages range from rounded to drop-shaped, with the presence of up to triple-pointed appendices in the ventral region. Presented low movements and lethargic periods.

The metanaupliar period encompasses stages N4 to N6, which develop over a duration of two to four days at 24 °C. During this phase, the metanauplii exhibit an elongated to fusiform morphology characterized by two maxillular exopods and rudimentary structures indicative of the formation of the first two swimming appendages in the ventral region. Presented rapid movements.

To ensure accurate differentiation between naupliar and metanaupliar stages, the first group of nauplii was harvested, followed by the second group two days later. This sequential culture method provided clarity in distinguishing between these developmental stages.

Adults were isolated individually by gender, males had rigid antennae bent from the eighth segment opposite the prosome, more elongated body than females, with 6 segments on the urosome, while females had mobile antennae folded towards the prosome, thicker body than males, with 5 segments on the urosome.

Filtration and ingestion experiment

The experiment to calculate the filtration and ingestion rates consisted of feeding *A. spartinus* with the microalgae *P. lutheri*. Each experimental unit consisted of 100 ml beaker with 25 ml marine water, these were distributed as follows: (a) three experimental units for nauplii (i-iii), (b) three experimental units for metanauplii (iv-vi) each one with concentrations of $3.0 \cdot 10^5$ cel/ml, (c) five experimental units for females and (d) five experimental units for males each one with concentrations of $1.4 \cdot 10^7$ cel/ml. There were blank samples without copepods for each group (Figure 1). Two aliquots were taken per sampling from each beaker ranging 3 h to 30 hours.

In all cases, a previous fasting period of 12 hours was considered (Betouhim-El & Kahan, 1972; Perga *et al.*, 2021); the aliquots were 1 ml and fixed with lugol, then the number of microalgae was counted in a

Neubauer camera under a Leica DM1000 LED microscope at 100X and corroborated at 400X. In no case were copepods dead during the period.

During the selection of the individuals, the appendages used to swim and trap prey cells were observed, as well as, the circular flow formed with these motile microalgae and in some nauplii the maximum distance moved in one second as a reference value with markings on the glass slide. Gonçalves & Kiørboe (2015) pointed that it is not clear how the way prey is perceived for non-motile microalgae otherwise they explain chemosensory aesthetascs and mechanosensory sensillas functions mainly in feeding appendages to recognize this kind of microalgae, the same functions could be applied to motile microalgae as *P. lutheri*.

Observation of the mandibular edge

Dissection of mandibular appendages presents challenges due to their small size and fragile structure, which complicates the observation of the mandibular edges. For this study, three adult females were randomly selected for the dissection of these oral structures using a Nikon SMZ 745 LED stereo microscope. The buccal parts were fixed between two 22x22 mm and 22x40 mm borosilicate glass plates with glycerine and 4% formaldehyde and sealed with DePeX-Gurr mounting media. The structures were observed on a Leica DM1000 LED microscope at 1000X magnification and photographed with a Leica DFC290 HD 3 megapixel CMOS image capture device. The cutting end of the mandible of each female was measured with Leica Application Suite software version 4.10.0. The images were printed, redrawn in as much detail as possible, scanned, and then turned to a vectorized illustration and edited in InkScape 0.92. Only three mandibles were measured correctly, due to their random position under the microscope.

The mandible edge index (MEI) proposed by Itoh (1970) was used to determine the feeding behavior of *A. spartinus*. Which was calculated from the cutting end measurements of the mandible of two females according to Formula 1. An edge index less than 500 suggests a predominantly herbivorous behavior (group I), between 500 and 900, an omnivorous behavior (group II) and one greater than 900, a carnivorous behavior (group III).

Drawing of the mandibular edge of the right mandible in ventral view of a female (Figure 2) shows the measured parts, being: V: Ventral tooth, C: Crested tooth of the mandibular edge, W: Total width of the mandibular edge, wi: Space between tips of each pair of teeth, H: Total height of the mandibular edge (with reference to the ventral tooth), hi: Height of each internal tooth and N: Total number of teeth on the mandible blade. The ventral tooth is generally the largest and usually varies between species, in this case its height served as a reference for the total height of the mandibular edge.

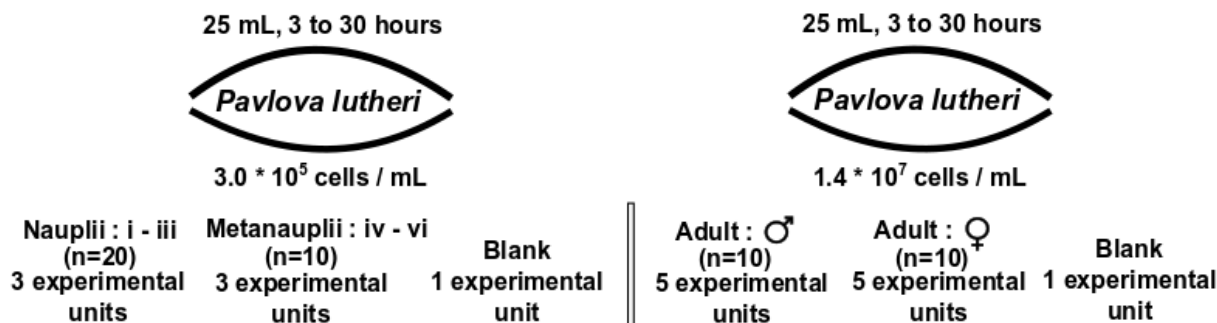


Figure 1. Experimental design.

$$MEI = \sum_{i=1}^{N=6} \left[\frac{(w_i)(h_i)}{6} 10^4 \right]$$

Formula 1

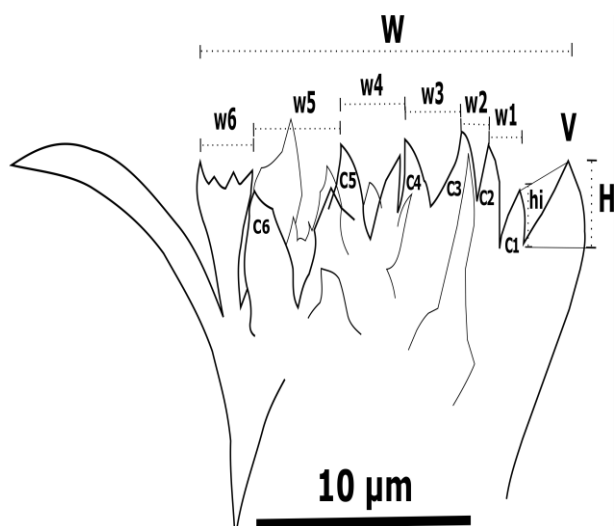


Figure 2. Diagram of the cutting end or mandibular edge of a female *Apocyclops spartinus*. The evaluated measurements are indicated by dotted lines.

Data collection

The following terms were defined in accordance with Frost (1972).

The microalgal growth constant (k) in the beakers with blank sample were calculated with Formula 2.

$$C_2 = C_1 e^{k(t_2-t_1)}$$

Formula 2

The copepod grazing coefficient (g) is obtained from the formula of microalgal growth in beakers with copepods (Formula 3).

$$C'_2 = C'_1 e^{(k-g)(t_2-t_1)}$$

Formula 3

Average concentration of microalgae in beakers with copepods (Formula 4).

$$[C] = \frac{C'_1 (e^{(k-g)(t_2-t_1)} - 1)}{(t_2 - t_1)(k - g)}$$

Formula 4

Where:

[C]: Average concentration of microalgae (cells/ml); C1: Initial food concentration in beakers without copepods (cells/ml); C2: Final food concentration in beakers without copepods (cells/ml); C'1: Initial food concentration in beakers with copepods (cells/ml); C'2: Final food concentration in beakers with copepods (cells/ml); t: Time (in hours); k: Microalgae growth constant and g: Copepod grazing coefficient.

Filtration rate. The volume of water that is separated from the suspended particles by the filtering apparatus from the organisms per unit of time (ml/hour). It is also known as the clearance rate.

$$F = \frac{Vg}{N}$$

Formula 5

Where:

F: Volume of water filtered under laboratory conditions (ml/individual hour); V: Volume of medium in beakers with copepods (ml) and g: Grazing coefficient; N: Number of copepods in the vessel.

Ingestion rate. The number of cells consumed by an individual organism in a specific time.

$$I = [C] F$$

Formula 6

Where:

I: Ingestion rate (cells/ individual hour); F: Filtration rate; [C]: Average concentration of microalgae (cells/ml).

Data were evaluated with the exponential and potential models in R software with their respective significance and graphic customization.

Results

Mandibular edge analysis

The appendages of the nauplii and adult copepods used for feeding were the antennulae, antennae, mandible, maxillae and maxillipeds, which cause small displayable circular flows, passively formed by microalgae that copepods then inspect to ingest or expel them. It was directly observed that naupliary stages, which have no swimming legs, used the antennulae and antennae to create the circular flow, their mandibles serve to propel them with rapid straight movements and can advance approximately one centimeter per second as its threshold, accompanied by vertical movements of the labrum, which occupies between the third and fourth part of the body. During the measurement process, the height of each tooth was determined according to its inner edge, as well as the distance between them; the sharp apex of each tooth was taken as a reference (Table 1). Itoh's mandibular edge index values greater than 900 were observed (Table 2), suggesting that *A. spartinus* has a selective feeding, although it strictly corresponds to the category of the carnivorous behavior.

Table 1. Measures in µm of width (W) and height (H) of the mandibular edge, from each of the six teeth (w[i] and h[i]) in females of *Apocyclops spartinus*.

		Female 1	Female 2	Female 3
	W	20.506	20.631	17.46
C1	w1	1.822	1.656	1.86
C2	w2	1.194	1.491	1.42
C3	w3	3.181	3.147	2.27
C4	w4	3.317	4.54	4.71
C5	w5	5.679	4.306	2.52
C6	w6	2.757	2.559	2.08
	H	3.648	3.678	4.42
C1	h1	2.121	2.353	3.28
C2	h2	3.446	3.644	3.37
C3	h3	2.981	2.619	2.31
C4	h4	2.418	2.187	3.46
C5	h5	4.34	5.116	3.54
C6	h6	4.853	4.582	3.89

Modeling of filtration and ingestion rates

Trends in ingestion rates per time (Formula 5) are reported as asymptotic curves, except in females that presented the highest initial consumption (Figure 3 d), but their fit in both models was not significant (Table 3), the highest intake was followed by males (Figure 3 c), metanauplii (Figure 3 b) and nauplii (Figure 3 a) which presented significance in the fit of both models (Table 3). Moreover, by modelling the data of combined females and males as one, the resulting intake curve named as adult became flattened

(Figure 3 e). The respective filtration rates per time (Formula 6 and Figure 4) had a similar trend to the ingestion rates. The highest filtration rate was presented by the metanauplii (Figure 4 b).

Table 2. Partial indexes of each crested tooth and index of the mandibular edge according to Itoh's formula (Itoh, 1970).

	Indexes		
	Female 1	Female 2	Female 3
Edge index 1	86.100	85.585	131.756
Edge index 2	91.671	119.336	103.348
Edge index 3	211.270	181.029	113.245
Edge index 4	178.696	218.083	351.949
Edge index 5	549.129	483.862	192.658
Edge index 6	298.099	257.538	174.741
Itoh's Index	1 414.965	1 345.434	1 067.696

Table 3. Models of ingestion rate for each essay of *Apocyclops spartinus* on *Pavlova lutheri*.

Stage	Model	R ²
Nauplii	$Y = e^{(182\ 334.7 - 0.452 X)}$	0.945 (*)
	$Y = 370\ 357.9 \times X^{(-1.88)}$	0.949 (*)
Metanauplii	$Y = e^{(373\ 423.2 - 0.262 X)}$	0.967 (*)
	$Y = 679\ 663.2 \times X^{(-1.256)}$	0.964 (*)
Males	$Y = e^{(1\ 767\ 404.6 - 0.088 X)}$	0.73 (*)
	$Y = 3\ 393\ 817.3 \times X^{(-0.733)}$	0.784 (*)
Females	$Y = e^{(1\ 521\ 720.1 - 0.024 X)}$	0.275 (ns)
	$Y = 2\ 074\ 708.7 \times X^{(-0.276)}$	0.297 (ns)
Adult (Females + Males)	$Y = e^{(1\ 512\ 365.996 - 0.04 X)}$ $Y = 2\ 442\ 908.08 \times X^{(-0.443)}$	0.413 (*) 0.451 (*)

Note. (*) Indicates that the determination coefficients are significant (p < 0.05) and (ns) indicates the non-significant ones (p > 0.05).

Gradual increases in the exponential curves superindexes were recorded up to levels close to zero, which explains that higher the stage of the copepod as a function of intake per time, it becomes exclusively dependent on Napier's constant (e), especially the values of the females or the combination of males and females. This last combination presented a better fit than the females alone, but less than the male model. Potential curves showed also gradual increases in the negative superindex scale, but with greater magnitudes than the exponential models. In both cases, the significance of each model was inverse with the ontogenetic development.

Discussion

In our study, consumption of *P. lutheri* is a consequence of its dynamism directly observed by its fast movement through flagella, smaller diameter, high lipid and protein content, 12.3 and 29.7 pg·cell⁻¹ respectively (Brown, 1991) besides it is considered an excellent source of long chain polyunsaturated fatty acids (Fábregas *et al.*, 2001).

Stoecke & Egloff (1987) demonstrated the predatory character of adults and nauplii of *Acartia tonsa* who consumed rotifers and ciliates, i.e., they maintained their preference for microzooplankton instead of the expected feeding in a microalgae-laden medium. In our case, the rapid movement of *P. lutheri* may imitate microzooplankton. It is relevant to regard, the effects of the formation of laminar currents by copepods to capture some microalgae, taking into consideration its buoyancy, through an active feeding path delimited by its chemoreceptors. This explains the reason for their efficient filtration rates and natural preference to develop in diluted environments (Strickler, 1982); this is also the case of *A. spartinus*, since it is very common to observe better population development with food concentrations below 10^7 cell/ml, in particular naupliary stages. Similarly, Wong (1988) demonstrates the influence of inter- and intraspecific interactions on grazing mechanisms by slowing down the speed of swimming or changing course to escape moving away from the food, such applies to nauplii of *A. spartinus*, which remain motionless most of the time, as a strategy not to be eaten by other copepods.

Berggreen *et al.* (1988) reported that the spectrum of detectable size of the microalgae is homogeneous from nauplius iv to adults of *A. tonsa* and tends to increase according to the stage considering only maximum filtration values. This assertion is reflected in our results with nauplii (i-iii) and metanauplii (iv-vi), since the latter presented higher activity and intake. Nauplii and metanauplii showed high filtration rates of *P. lutheri* during the first three hours, a higher energy expenditure is assumed to obtain its food from starvation and there is a marked balance after 24 hours.

Copepod nauplii exhibited long periods of drift on, along with prey-reflex movements as a defensive mechanism in response to predator presence, this behavioral adaptation not only aids in evading predation but also facilitates opportunistic feeding on microalgae that come into close proximity during these reflexive movements (Titelman & Kiørboe, 2003). Such feeding strategies are crucial for their growth and development, allowing them to capitalize on available food resources while minimizing exposure to predators (Wilson, 1973). As noted by Helenius & Saiz (2017), the ability of nauplii to engage in both evasive and feeding behaviors simultaneously underscores the complexity of their ecological interactions and the importance of microalgal availability. Unlike nauplii, the attack jumps in ambush feeding metanauplii have a greater displacement, also body volume (Bruno *et al.*, 2012), so their filtration rate was the highest throughout the experiment, which may explain the natural cause of mortality in nauplii, due to inefficiency in capturing their food, in particular at 24 °C the first 16 to 24 h,

whose *A. spartinus*' nauplii development consume its oil droplet reserves observed.

Metanauplii presented at the beginning a high filtration like the females; in contrast, they had very low values at 24 h, suggesting a high energy cost of feeding, which is another natural cause of mortality in oligotrophic conditions or in the colonization of a new habitat. This feeding behavior coincides with that indicated by Paffenhöfer *et al.* (1996), who reported that marine cyclopoid nauplii are slower than calanoids.

The individual ingestion rate essay showed that *P. lutheri* consumption is higher in females, 1.5 times than males, 18 times than metanauplii and 75 times than nauplii. This explains the allometric relationship between predator and prey, as stated by Giesecke & González (2004), which influenced the efficiency of capture during foraging, this author also suggests supplementing feeding essays on copepods with morphological studies of the mandibular border to corroborate the Itoh index. This suggestion was evaluated using measurements of the mandibular edge of three female of *A. spartinus* to characterize their active ambush predatory strategy, aligning with the classification within the carnivorous spectrum as defined by Itoh's index, moreover it is outlined the use of feeding appendages for nauplii and adult copepod. Furthermore, *A. spartinus* cultures decrease when fed with *Nannochloris* or *Nannochloropsis*, as it was also shown by Velásquez *et al.* (2001) when low population growth values were obtained for *Apocyclops distans* fed with *Nannochloris oculata*. Gonçalves *et al.* (2012) reported a strategy of seasonal dietary change between diatoms and dinoflagellates, but not smaller cells like Chlorophytas. We took these ideas to add feeding behavior, regarding specific mandibular morphology, as a new complementary factor to the microalgae's lipid reserves, which is sometimes the only consideration for feeding copepods.

Under this method of synchronized pre-fasting, the models explain the intake of the microalgae *P. lutheri* as a function of time and by groups of stages of *A. spartinus*. The reliability of these two models was evaluated and compared with seven other non-linear models (exponential decline, logistics, natural logarithm, modified power, modified exponential, root and yield-spacing reciprocal), so the mathematical conceptualization fulfills the purposes of predicting food intake and is also friendly for future applications (Tedeschi, 2006). However, the determination coefficient (R^2) was lower in adults, explained by the higher consumption that generates more dispersion of data. In both models the superindexes are negative close to zero and the females presented the lowest magnitude, which implies a higher rate of ingestion as a result of a very small denominator that magnifies their consumption compared to males and naupliary groups (Table 3).

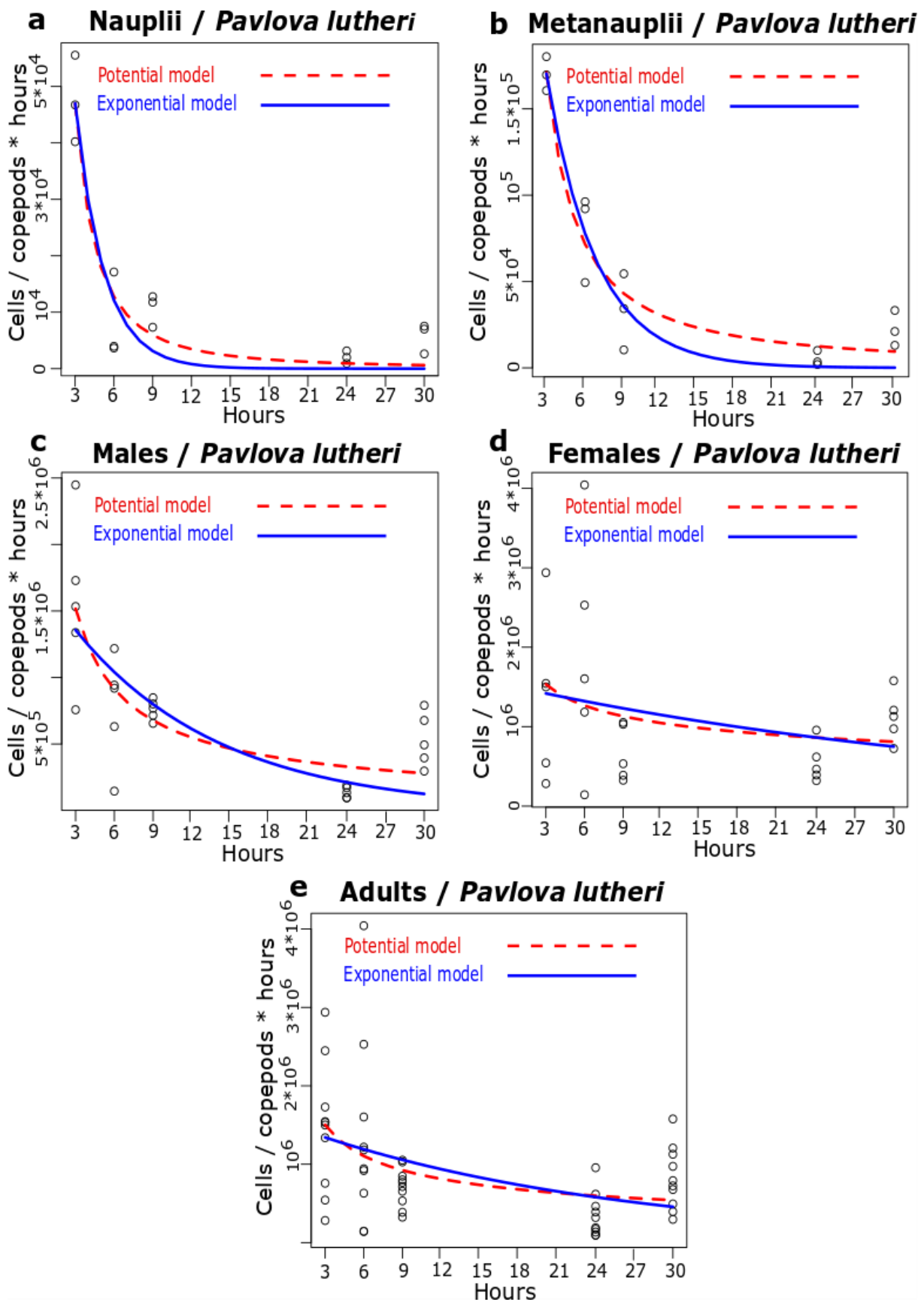


Figure 3. Ingestion rate of *Apocyclops spartinus* on *Pavlova lutheri* cultures at 24 °C.

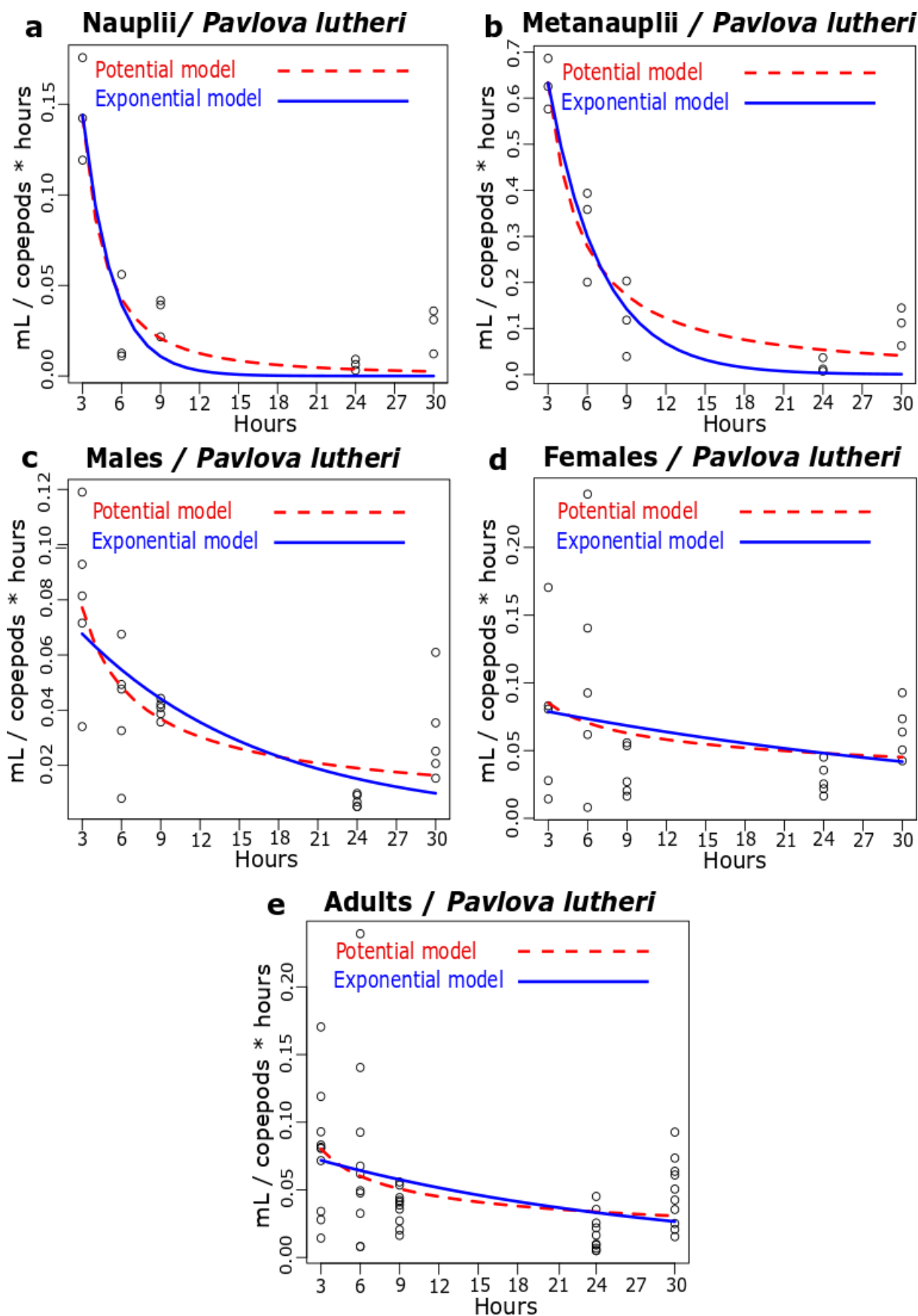


Figure 4. Filtration rate of *Apocyclops spartinus* on *Pavlova lutheri* cultures at 24 °C.

No mortality was evident during the study period. The females presented a continuous search for food related to high filtration values, due to their inherent metabolism of pre reproductive nutritional requirements, as well as for egg formation and spawning (Besiktepe & Dam, 2020) (Figure 4 d). This voracity also implies greater energy expense by filtering more water in an initial state of starvation and showing more dispersed initial values with a clear negative asymptotic tendency that tend to a small increase after 24 h. Males have a random copulative role, so their feeding is smaller, more homogeneous and efficient, since it is not constant (Figure 4 c). The latter have a more elongated morphology, which promotes faster swimming and their filtration tendency increased after 24 h. These differences were also pointed out by van Someren Gréve *et al.* (2017) when studying the feeding habits of male and female *Oithona nana*.

These synchronized feeding experiments can be very useful in live food production chains, in toxicological tests or in maintenance for preservation, thus avoiding food saturation or shortage that may influence an applied treatment, in which the metabolism of the copepod is required to be optimal to evaluate a variable of interest.

Conclusions

The predatory behavior of *A. spartinus* was corroborated with the Itoh's mandibular edge index feeding on motile microalgae such as *P. lutheri*.

Five exponential and five potential models were applied to describe ingestion and filtration feeding behavior after 12 hours of fasting for the maintenance of *A. spartinus*. Model adjustments were significant with the exception of the female. The uses of these two models are proposed to be reliable and friendly application. The superindexes at the exponential level were more negative in the naupliary stages compared to the adults. In the case of adults, the females presented the values closer to zero, which explains that function of intake per time at the ontogenetic level becomes almost exclusive of Napier's constant (e). Both models are consistent with the higher consumption of females with respect to the naupliary stages.

The negative trend of these superindexes, showed by an asymptotic curve, was related to the satiety of the copepods. In addition, these parameters explain the higher demand by adults over naupliary stages, it also correlates with the higher rate of intake of females typical of the energy requirement for the formation of eggs and embryos. Although other more complex non-linear models could present a better fit with other microalgae or in other laboratory conditions, the interpretation of their parameters would limit their widespread use, which would contrast with the models used in the present work.

The results under this method allowed us to observe that during the initial hours, food was used at

maximum, which corresponds to the high rates of ingestion and filtration reported at the beginning of the trials. Therefore, the less steady intake at the end of each experiments was due to the low quantity of remaining food, its quality and satiety of the copepod, which is why these essays justify their short duration.

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Conflict of interest

There is no conflict of interest nor potential competing interest was reported by the authors.

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Appendix 1. Average mixed ingestion of *A. spartinus* expressed in percentages.

Mixed ingestion averages (cells/individual hour) at 24 °C

Time	<i>Pavlova lutheri</i> [3.0·10 ⁶ cel/ml]	<i>Tetraselmis suecica</i> [3.5·10 ⁵ cel/ml]	<i>Skeletonema costatum</i> [3.1·10 ⁵ cel/ml]	Ingestión total %
24 hours	(53.56 %)	(18.11 %)	(28.33 %)	37.92 %
48 hours	(84.22 %)	(4.87 %)	(10.91 %)	38.48 %
72 hours	(89.96 %)	(1.85 %)	(8.19 %)	23.60 %

Feeding *Nannochloropsis* spp. or *Nannochloris* spp. resulted in markedly stunted population growth and high mortality in *A. spartinus*. We deduce that this copepod does not use them as food. So it is unfeasible to assess their consumption. (taken from the first author's master's thesis-unpublished).

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