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Ciliate (*Euplotes* sp.) predation by *Pseudodiaptomus annandalei* (Copepoda: Calanoida) and the effects of mono-algal and pluri-algal diets

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Abstract

Background: *Pseudodiaptomus annandalei* is an estuarine species and being cultured as live feed for grouper fish larvae and other planktivores. We examined the predation behavior of *P. annandalei* adults when preying on ciliated protists (*Euplotes* sp.) and the effects of mono- and pluri-algal diets on ciliate predation by *P. annandalei* under laboratory conditions. The algal food comprised the pigmented flagellate *Isochrysis galbana* (4 ~ 5 μm) and *Tetraselmis chui* (17 ~ 20 μm).

Results: Males and females of *P. annandalei* consumed 8 ~ 15 ciliate cells/h. The probability of ciliate ingestion following an attack was a direct function of the copepod's hunger level. Conversely, the probability of prey rejection after capture was a negative function of the copepod's hunger level. Starved and poorly fed females showed a significantly lower rate of prey rejection compared to similarly treated males. The duration of handling a ciliate prey did not significantly differ between males and females of *P. annandalei*. Starved copepods spent less time handling a ciliate prey than fed copepods. Prey ingestion rates showed a negative relation with the feeding duration, whereas the prey rejection rate increased as the feeding duration increased. The ciliate consumption rate of *P. annandalei* was significantly lower in the presence of mixed algae. Neither *I. galbana* nor *T. chui* alone had any significant effect on ciliate consumption by *P. annandalei*.

Conclusions: The results confirmed that *P. annandalei* ingests bacterivorous heterotrophic protists even in the presence of autotrophic protists. Therefore, our results point to the role of *P. annandalei* in the transfer of microbial carbon to the classical food chain in estuarine and brackish water ecosystems.

Keywords: Copepod, Ciliate, Algal prey, Feeding, Predation, Hunger level

Background

Copepods play a central role in transferring carbon energy from lower trophic levels to higher trophic levels such as fish in estuarine and marine food webs (Turner 2004). *Pseudodiaptomus annandalei* is a euryhaline species found perennially in coastal, estuarine, and brackish waters in the tropical and subtropical Indo-Pacific (Madhupratap 1987; Hwang et al. 2010; Kâ and Hwang 2011; Dahms et al. 2012; Dur et al. 2012). Recent laboratory studies and the

perennial occurrence of this species in nature indicate that *P. annandalei* can utilize a wide spectrum of food (Hwang et al. 2010; Dhanker et al. 2012). In turn, this species constitutes a major portion of the diet of numerous estuarine fish larvae. Its mass culture is also being carried out, and it is currently being used as live feed in larviculture of grouper fish larvae and other planktivores (Doi et al. 1997; Hagiwara et al. 2001; Liao et al. 2001; Chen et al. 2006; Lee et al. 2010; Celino et al. 2012). *P. annandalei* was traditionally considered to be herbivorous and is commercially produced on a microalgal diet by the aquaculture industry. The mixotrophic and predatory nature of this species was recently shown by Cheng et al. (2011) and Dhanker et al.

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(2012). The feeding ecology of this species has been well documented on some commonly available microalgae (Liao et al. 2001; Chen et al. 2006; Beyrend-Dur et al. 2011) and rotifers (Dhanker et al. 2012). No information is as yet available on the feeding potential of *P. annandalei* on ciliate protists.

Ciliates were identified as a major food source of copepods in detritus-rich estuaries with lower chlorophyll levels (Landry et al. 1993; Atkinson 1996; Vincent and Hartmann 2001). Numerous studies proved that copepods can utilize considerable proportions of ciliate production (Kumar 2003; Huo et al. 2008; Fileman et al. 2010). Ciliate consumption rates by certain copepods were significantly higher than those for algae (Gifford and Dagg 1988; Vadstein et al. 2004; Loder et al. 2011; Küppersm and Claps 2012), and clearance rates for ciliates were equal as they cleared large algal cells (Tiselius 1989; Gifford and Dagg 1991). Because they can exploit food stocks more efficiently due to their higher metabolism and growth rates (Hansen 1992, Sherr and Sherr 2007), heterotrophic protists have a higher biomass conversion potential compared to autotrophic protists (Gifford 1991; Broglio et al. 2003; Tang and Taal 2005) and are more nutritionally important qualitatively and quantitatively in diets of copepods (Adriana et al. 2006). Therefore, ciliates may constitute an important food source for *P. annandalei* in nature where primary production is scarce. Ciliates may convert lipids obtained from their food to polyunsaturated fatty acids (PUFAs) (Sul and Erwin 1997) which are essential for copepod growth and reproduction. Ismar et al. (2008) reported that the estuarine copepod *Acartia tonsa* showed a behavioral preference for *Euplotes* over diatom feeding.

Copepods require sterols as an essential component for their growth and egg production (Ederington et al. 1995; Klein Breteler et al. 1999), and these are synthesized by the copepods themselves. Cholesterol synthesis was detected in the hypotrich ciliate protist *Euplotes* sp. (class Nassophorea) (Harvey and McManus 1991).

The feeding mechanism of copepods in nature is strongly influenced by the availability of alternate food sources (Kumar 1999a, b; 2003) and by the motility, density, size, age, reproductive stage, and abundance of prey (Jonsson and Tiselius 1990; Rao and Kumar 2002; Jakobsen et al. 2005; Tseng et al. 2009; Dhanker et al. 2012). Other factors are hunger level, satiation, age, and sex of the copepods (Salvanes and Hart 1998; Kumar and Rao 1999a, b; Asaeda et al. 2001; Rao and Kumar 2002; Kumar 2003; Dhanker et al. 2012). The ciliate, *Euplotes* sp., often coexists with *P. annandalei*, and it may be a preferred food source for copepods when primary production is limited. However, no information is available about *P. annandalei* predation on ciliate protists. The aim of this study was to understand the details of the ecological and behavioral attributes of *P. annandalei* predation on a ciliate protist (*Euplotes* sp.). We attempted to answer the following questions through this study: (a) Can *P. annandalei* utilize heterotrophic ciliate protists as a food source? (b) To what extent does the presence of autotrophic protists in its environment affect predation on heterotrophic protists?

Methods
Experimental organisms
Starter cultures of adult *P. annandalei* were isolated from zooplankton samples collected from a coastal brackish water pond of Taiwan. A monoculture was developed in a mixture of filtered seawater and autoclaved tap water, and this was inoculated into a 5-L aquarium that contained 4 L of medium. A mixture of the microalgae *Isochrysis galbana* and *Tetraselmis chui*, rotifer *Brachionus rotundiformis*, and ciliate *Euplotes* sp. was used as food for the copepods (Table 1). The culture was maintained at a salinity of 20 psu and 28°C under a photoperiod of 12 h of light and 12 h of dark. The copepod culture was maintained in the laboratory for ≥3 months prior to the experiment. Moreover, ≥200 ovigerous females of *P. annandalei* were collected to obtain freshly hatched nauplii to perform the experiment. All experiments were conducted with *P. annandalei* of a known age (18 to 20 days). The culture was continuously mildly aerated to keep the food uniformly distributed in the culture tank. The culture medium was renewed twice a week.

Ciliates of the genus *Euplotes* were originally isolated from the rotifer culture tank. They were propagated and maintained in a 2-L beaker at a salinity of 20 to 25 psu and fed to the unicellular alga *I. galbana* (Table 1). The abundance of *Euplotes* cells was determined using an inverted microscope. The culture medium was changed on alternate days with a mixture of filtered autoclaved seawater and autoclaved tap water (20 to 25 psu).

Mass cultures of both algal species (*I. galbana* and *T. chui*) were established in the laboratory. Algal culture media were prepared by enriching sterile filtered seawater with macronutrients and micronutrients (Walne medium; Walne 1970) in a 2-L borosilicate glass flask. Individual monocultures of both algae were maintained at a salinity of 20 psu at a photoperiod of 12 h of light and 12 h of dark. The algae were harvested in their exponential growth phase of the nutrient-replenished condition to prevent mineral nutrient limitation. Details of the experimental organisms are shown in Table 1.

Experimental protocol
All experiments were conducted at a salinity of 20 psu and at a fixed temperature (28°C) in BOD (biochemical oxygen demand). Experiments were conducted in three consecutive phases: (a) the effects of algal diets on ciliate consumption rates by males and females of

Table 1 Experimental organisms, their body sizes, and culture conditions

Ecological group	Taxonomic group	Species	Body size (μm)	Culture condition
Predator	Copepoda	*P. annandalei* female	1,280 ± 56	Mixture of autoclaved seawater and tap water with algae, rotifers, and ciliates as food
Predator	Copepoda	*P. annandalei* male	1,086 ± 78	Mixture of autoclaved seawater and tap water with algae, rotifers, and ciliates as food
Prey	Ciliophora	*Euplotes* sp.	46 ± 8	Mixture of autoclaved seawater and tap water with *I. galbana*
Prey	Chromalveolata	*I. galbana*	4.16 ± 0.65	Walne's medium (Walne 1970)
Prey	Chlorophyta	*T. chui*	17.35 ± 1.98	Walne's medium (Walne 1970)

P. annandalei, (b) ciliate ingestion in relation to the satiation level of copepods, and (c) elucidation of the act of ciliate capture by males and females of *P. annandalei*.

Effects of algal diets on ciliate consumption rates

Predation rates on ciliates by males and ovigerous and nonovigerous females of *P. annandalei* were examined in the presence and absence of an algal diet. The experimental protocol included the following: (a) ciliate prey alone, (b) ciliates with *I. galbana*, (c) ciliates with *T. chui*, and (d) ciliates with *I. galbana* and *T. chui*. Known-age individuals of *P. annandalei* were collected from stock cultures and transferred to a bowl containing 40 mL of medium 3 h prior to the experiment. *P. annandalei* was deprived of food for 3 h prior to the experiment. Subsequently, 40 cells of *Euplotes* sp. were introduced into each bowl, and five bowls were used for each treatment. The number of consumed cells was recorded after 60 min. *P. annandalei* was removed from the experimental bowl at the end of the test, and all remaining live prey from each bowl were carefully counted under a zoom stereomicroscope (Olympus SXZ 16; Shinjuku, Tokyo, Japan) to obtain an estimate of the number consumed.

Feeding by *P. annandalei* as a function of satiation time and satiation level

The starting time for feeding by an animal to a voluntary pause despite food availability is considered the satiation duration. In total, five *P. annandalei* females (18 to 20 days old) were collected from the stock culture, individually placed in a 50-mL experimental bowl with 40 mL of water medium, and deprived of food for 3 h to estimate their satiation duration. Thereafter, 40 (1 ciliate individual (ind)/mL) ciliates were introduced into each experimental bowl containing *P. annandalei*. The number of consumed ciliates within 15 min was recorded as the first observation, and prey consumption was subsequently recorded at 30-min intervals. The number of ingested cells in 30 min was replaced with fresh cells to maintain a constant prey concentration (number of ciliates = 40/experimental bowl).

Predation efficiency (microscopic observation) as a function of hunger level

The predation behaviors of male and female *P. annandalei* on *Euplotes* sp. were examined in this experiment. The number of predatory steps such as prey searching, handling time, post-attack prey ingestion, and rejection probabilities in relation to the hunger level was recorded during the experiment. Well-fed, poorly fed (starved for 30 min), and starved (starved for 2 h) copepods were used for the experiment. Copepods were acclimated for 15 min in an experimental glass cavity block (4.5-cm diameter and 1.7-cm-deep petri dish) containing 20-mL medium at 20 psu. Moreover, the required number of ciliate cells was counted and stored in a separate petri dish. *P. annandalei* predation behavior was directly observed for 15 min (using a stopwatch) in five replicates for each treatment under a zoom stereomicroscope (Olympus SXZ 16). Observations were performed on a glass cavity block (3.2-cm diameter and 0.6-cm-deep petri dish) containing 5 mL of medium.

The probability of prey ingestion after an attack (PI) and probability of prey rejection after an attack (PR) were calculated using the following formulae:

$$\text{PI} = \text{mean no. of ingestions/mean no. of attacks and}$$
$$\text{PR} = \text{mean no. of rejections/mean no. of attacks}$$

The differences in the probability of each predation event were analyzed using a stepwise analysis of variance (ANOVA); probability data were arch-sine-transformed for statistical analyses.

Prey rejection as a function of feeding duration

Prey ingestion and rejection rates were observed in this experiment for differentially starved *P. annandalei* individuals. Female individuals were allowed to feed on ciliates for various durations prior to initiating the experiments. Experiments were conducted in a transparent glass cavity block (3.2-cm diameter and 0.6-cm-deep petri dish) containing 5 mL of medium. Thereafter, 20 cells of *Euplotes* sp. were introduced into the cavity block, and each ingestion and

rejection event of ciliate cells was carefully recorded under a microscope for every 15-min interval.

Results
Direct microscopic observations proved that *P. annandalei* actively consumed *Euplotes* cells. First, *P. annandalei* used filter feeding currents to bring ciliates near its mouthparts and when ciliate prey approached an appropriate capturable distance, the copepod captured and ingested them. Images of female *P. annandalei* capturing cells of *Euplotes* sp. are shown in Figure 1.

Effects of algal diets on ciliate consumption rates
Both male and female (nonovigerous and ovigerous) *P. annandalei* efficiently ingested *Euplotes* cells (Figure 2). Gender-based differences in ciliate ingestion by *P. annandalei* were not significant ($p > 0.37$, one-way ANOVA), and 8 to 15 *Euplotes* cells/h were consumed. Male and female *P. annandalei* showed differential responses to an algal-diet presence in the medium. Neither *I. galbana* (a smaller alga) nor *T. chui* (a larger alga) alone showed any significant effect ($p > 0.05$, one-way ANOVA, Figure 2) on ciliate consumption by ovigerous and nonovigerous *P. annandalei* females. However, the presence of *I. galbana* elicited significantly ($p = 0.018$) less ciliate ingestion by *P. annandalei* males. Furthermore, the combination of the smaller and larger algae elicited significantly lower ciliate consumption rates ($p < 0.05$, one-way ANOVA) by both male and female *P. annandalei* (Figure 2).

Feeding by *P. annandalei* as affected by satiation time and satiation level
P. annandalei females efficiently cleared *Euplotes* cells for 105 min in a food-rich environment. Although copepods were kept in a food-rich environment for 225 min, the number of cells consumed did not significantly differ from 105 to 225 min. Therefore, the consumption of ciliates by female *P. annandalei* reached a satiation level at 105 min, after ingesting a total of 25.5 ± 2 *Euplotes* cells (Figure 3).

Predation efficiency (microscopic observations) as a function of hunger level
Through repeated observations using a zoom stereomicroscope, *P. annandalei* was shown to react to ciliates that approached its visual horizon, outside of which no reaction was observed independently of the distance from the first antennae. *P. annandalei* created feeding currents that carried prey towards its capture zone, bringing ciliates near its antennules. Prey handling, ingestion, and rejection were observed. Prey capture events were analyzed using our behavioral observations.

Prey searching and handling times
Prey searching times were significantly influenced by the hunger state of the predator. The hunger level exerted a significant effect ($p = 0.002$, one-way ANOVA, Figure 4A) on *P. annandalei* males searching for prey, but was not significant ($p = 0.35$, one-way ANOVA) in female calanoids (Figure 4A). Well-fed males required a significantly longer duration ($p < 0.05$) to search for prey compared to their poorly fed and starved counterparts. However, prey searching times did not significantly differ between poorly fed and starved males. Prey searching times were significantly longer ($p < 0.05$, Student's *t* test) in well-fed and poorly fed *P. annandalei* males compared to female calanoids (Figure 4A). Hunger level related differences in prey handling times did not significantly differ ($p = 0.152$; Figure 4B) in female *P. annandalei*, but significantly differed ($p = 0.005$, one-way ANOVA; Figure 4B) in males. The prey handling time for starved males was significantly lower ($p < 0.001$) than those of well-fed and poorly fed male *P. annandalei*. Furthermore, prey handling times were significantly longer ($p < 0.05$) in males than females.
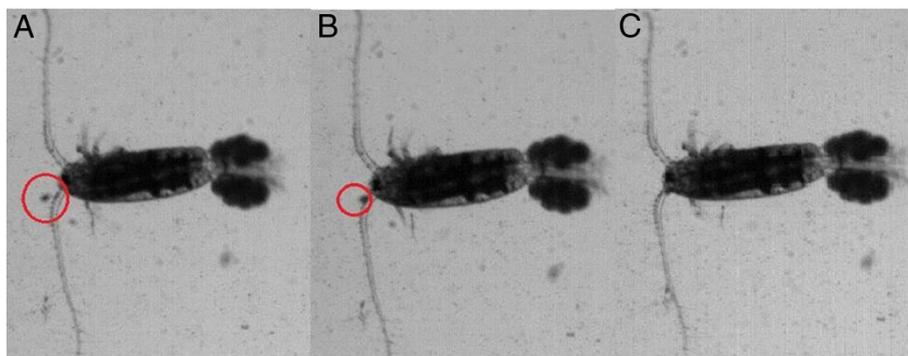
Figure 1 Images of female *P. annandalei* pursuing (A and B) and ingesting (C) the ciliate *Euplotes* sp. The red circle indicates the presence of ciliate prey before ingestion.

Figure 3 *Euplotes* cells consumed by females *P. annandalei* to reach satiation level in relation to different feeding durations.

Figure 2 Effects of algal diets on ciliate consumption (mean ± SE). (A) Ovigerous females, **(B)** Nonovigerous females and **(C)** Males of *P. annandalei*. I. g = *I. galbana*, T. c = *T. chui*.

Figure 4 Prey searching and handling times by males and females of *P. annandalei*. Prey searching time **(A)** and handling time **(B)** (seconds; mean ± SE) by males and females of *P. annandalei* as a function of the hunger level. Significant differences are indicated by letters within a gender and by asterisks between genders.

Post-attack probabilities of ingestion and rejection

Probabilities of cell ingestion significantly increased ($p < 0.001$, one-way ANOVA; Figure 5A) with increasing hunger levels in both male and female *P. annandalei*. Prey ingestion probabilities were significantly higher ($p < 0.05$, Student's t test; Figure 5A) in poorly fed and starved females compared to male calanoids. Differences in prey ingestion probabilities were not significant ($p = 0.117$, Student's t test) between genders for well-fed *P. annandalei*.

Figure 5 Post-attack probabilities of *Euplotes* cell ingestion and rejection. Post-attack probabilities of *Euplotes* cell ingestion **(A)** and rejection **(B)** (mean ± SE) by males and females of *P. annandalei* with an increasing hunger level (well fed, poorly fed and starved). Significant differences are indicated by letters within a gender and by asterisks between genders.
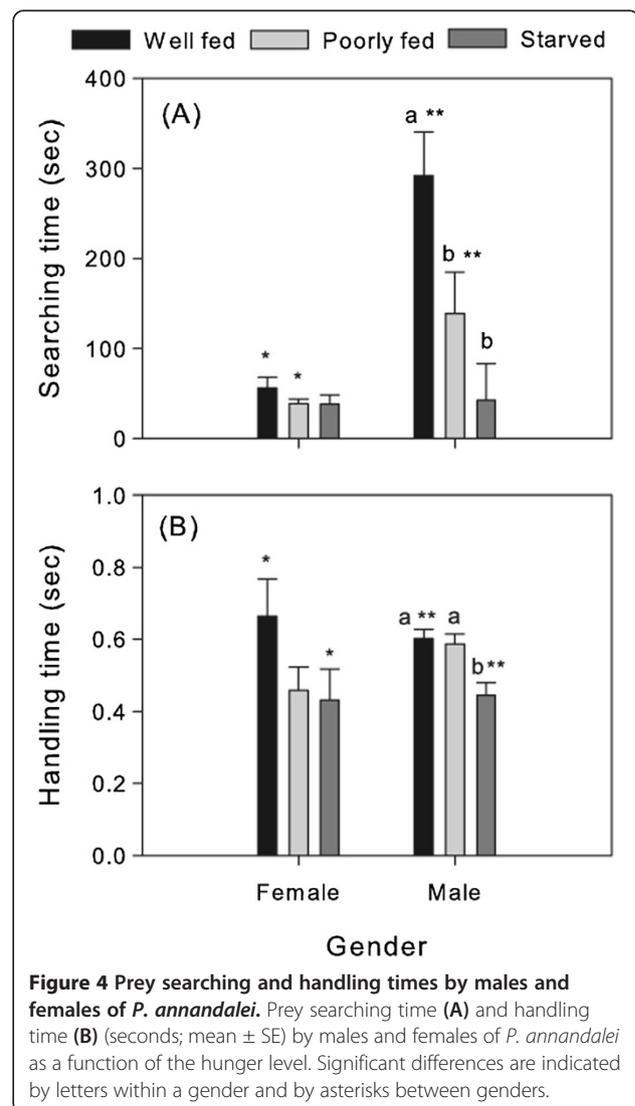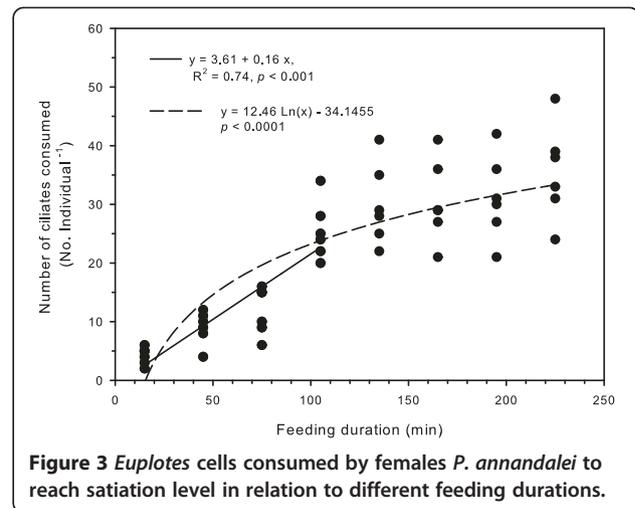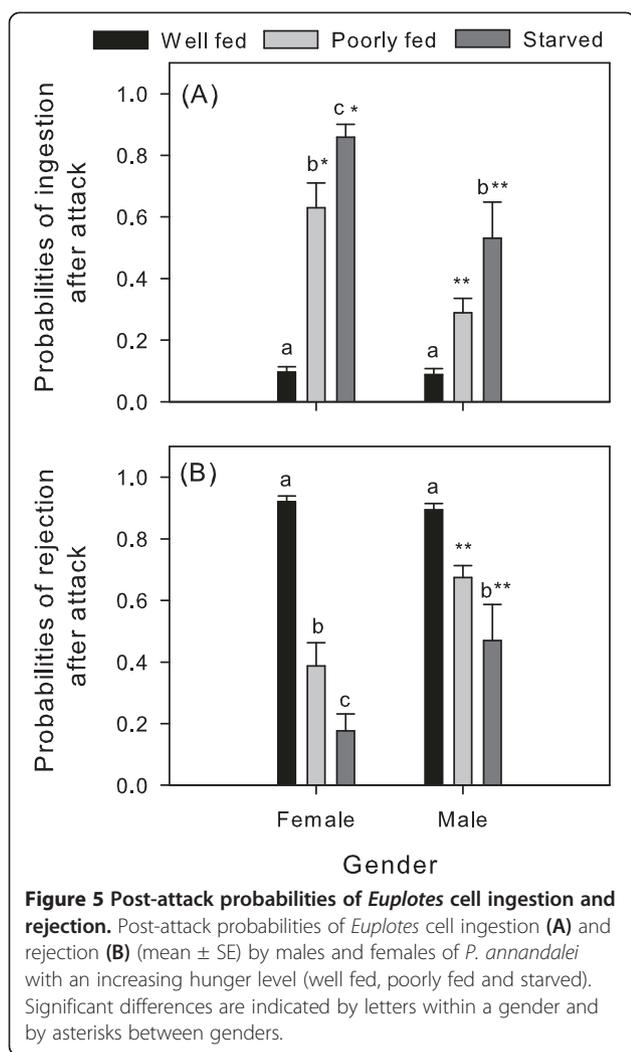
Prey rejection probabilities were inversely proportional to the hunger level of *P. annandalei*. Prey rejection frequencies were significantly higher in poorly fed and starved male calanoids than in the same female calanoids ($p < 0.05$), but there was no significant difference for well-fed calanoids ($p = 0.82$, Student's t test; Figure 5B). The highest prey rejection probabilities were recorded ($p < 0.0001$, one-way ANOVA; Figure 5B) for both female and male *P. annandalei* that were starved for the longest duration (i.e., at the maximum hunger level).

Furthermore, prey ingestion and rejection were inversely correlated with the feeding duration in female *P. annandalei*. The number of rejected ciliate cells was significantly higher ($p = 0.003$, $R^2 = 0.91$), and cell ingestion was significantly lower ($p = 0.001$, $R^2 = 0.98$; Figure 6) at the longest feeding duration in female calanoids.

Discussion

Visual behavioral studies in laboratories provide reliable quantitative information regarding the sequence of feeding steps, such as prey searching, handling times, attack, capture, ingestion, and rejection by a predator (Trager et al. 1990; Awasthi et al. 2012a), efficiency of a predator in capturing prey (Hwang and Strickler 2001; Rao and Kumar 2002; Dhanker et al. 2012), and antipredator (escape) behavior by prey (Awasthi et al. 2012b). Dhanker et al. (2012) indicated that *P. annandalei* fed efficiently on the rotifer *B. rotundiformis* Tschugunoff, 1921 even in the presence of alternate algal food. This study further elucidated the predatory behavior of *P. annandalei* on a heterotrophic ciliate. The ciliate genus used in this study is widely distributed (cosmopolitan) such as several other microzooplankton. The contribution of ciliates to food sources of copepods is commonly known for freshwater (Wickham 1995; Reiss and Schmid-Araya 2011; Kamjunke et al. 2012), estuarine (Wiadnyana and Rassoulzadegan 1989; Vincent and Hartmann 2001), and marine ecosystems (Levinsen et al. 2000; Calbert and Saiz 2005). A mixture of ciliates and phytoplankton provided different results from that of either prey alone (Kumar 2003). Numerous studies showed higher clearances of ciliates over algal food, but several showed equal ingestion rates. Conversely, lower ciliate ingestion rates were verified (see reviews by Stoecker and Capuzzo 1990; Sanders and Wickham 1993).

In this study, *P. annandalei* efficiently ingested *Euplotes* cells, and *Euplotes* cell consumption was not affected by the mono-algal diets. The presence of a pluri-algal diet significantly affected ciliate consumption by male and female *P. annandalei*. This study concluded that *P. annandalei* exhibited a behavioral preference for ciliated protists. It should be noted that marine calanoids require ω3-PUFAs in their food for growth and development (Brett and Müller-Navarr 1997), and hence any food containing ω3-PUFAs is considered a nutritionally enriched diet for copepods. The present study did not discuss the nutritional contents of ciliate protists for *P. annandalei*. The ciliate prey tested in this study, *Euplotes* sp. (Zhukova and Kharlamenko 1999), was reported to possess the ability to synthesize highly unsaturated fatty acids (HUFAs) (Mieczan 2012). Moreover, *Euplotes* sp. is the only ciliate known to produce a sterol (Harvey and McManus 1991). Sterols are indispensable for maintaining cell membrane fluidity (Ourisson et al. 1987); thus, they occur in nearly all higher organisms. Although *P. annandalei* produced eggs on microalgal diets in culture conditions, we presumed that adult *P. annandalei* would be able to produce more eggs on a mixed diet of ciliate and algae than solely on a pure algal diet.

The predatory feeding behavior of *P. annandalei* was recently experimentally described (Dhanker et al. 2012), and this is the first study to demonstrate *P. annandalei* predation on a ciliated protist. In aquaculture practice, numerous species of the genus *Pseudodiaptomus* have been cultured on algae alone (Pagano et al. 2003; Puello-Cruz
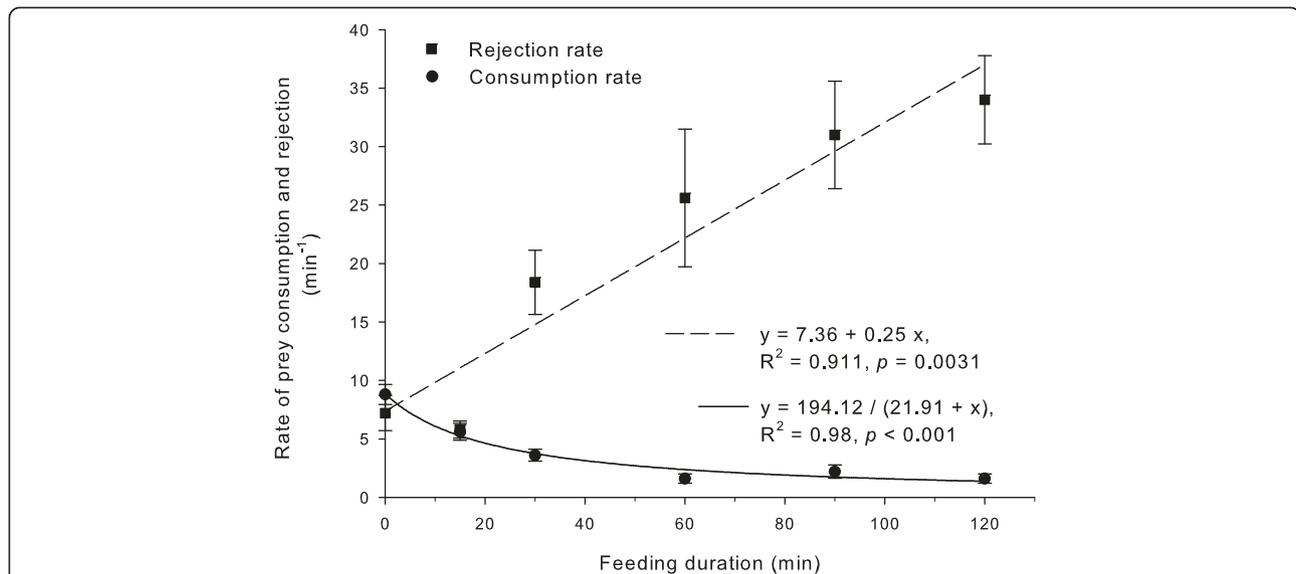
Figure 6 *Euplotes* **cell ingestion and rejection (mean ± SE) by female** *P. annandalei* **in relation to feeding duration.**

et al. 2009; Ohs et al. 2010). This suggests that truly herbivorous or truly carnivorous taxa are rare. Our observations suggest that adult *P. annandalei* can consume heterotrophic ciliates even in the presence of algal food. Calanoid copepods are generally more effective in controlling ciliate communities than cyclopoids (Burns and Gilbert 1993; Wickham 1995). Even smaller diaptomids have the potential to control both the biomass and species structure of ciliate communities (Burns and Gilbert 1993). The omnivorous calanoid, *Acartia*, had a significantly higher clearance rate of ciliate prey than of phytoplankton (Gifford and Dagg 1988). Similarly, in the Bay of Biscay, *Temora longicornis* and *Centropages chierchiae* were found to ingest ciliates at higher rates rather than chlorophyll pigments (Vincent and Hartmann 2001).

Two algal species were used as alternative food in this study (Table 1). The microalga *I. galbana* is at the smaller size limit of the dietary niche width for calanoid copepods. Copepods generally avoid food of <5 μm in size regardless of their body size (Pagano et al. 1999, 2003; Fileman et al. 2007; Jang et al. 2010). Larger protozoa (>10 μm in length or width) are ingested at high rates rather than phytoplankton by various species of calanoid copepods in various stages (Ohman and Runge 1994). This study demonstrated that *P. annandalei* (a) exerted strong predation pressure on ciliated protists and (b) actively fed on ciliates in the presence of either smaller alga *I. galbana* or larger alga *T. chui*, but the feeding activity was differentially influenced by the algal species and their combination.

In this study, longer searching and handling times, and lower ingestion and higher rejection rates of ciliates in male *P. annandalei*, suggest that males are less efficient predators compared to their female counterparts. Behaviors of ciliates belonging to the genus *Euplotes* following exposure to predator cues were extensively described (Kusch 1993b; Altwegg et al. 2004; Duquette et al. 2005). *Euplotes* cells were observed to respond to the predatory flatworm by producing large lateral wings, a dorsal ridge, and ventral projections, as well as significantly increasing their maximum body width (Wiackowski and Staronska 1999; Altwegg et al. 2004). These morphological changes lead to a significantly reduced probability of ingestion by predators (Kuhlmann and Heckmann 1994). Moreover, changes in *Euplotes* behaviors following exposure to amoeboid predators were previously documented (Kusch 1993). Predator encounters are associated with a prey's movements, indicating that a reduced velocity during foraging may be an adaptive measure because it lowers the chances of encountering predators. Therefore, observing each event of prey capture and the probability of successive events during prey capture is important in understanding prey capture success.

The probability of each event following preceding events during predation in copepods is variable and dependent on biotic and abiotic factors. Copepods in nature regularly experience hunger because of long-term seasonal changes in vertical migrations of the food supply (Runge 1980) and/or prey patchiness (Dagg 1977). Hunger levels have important roles in feeding behaviors of copepods (Runge 1980; Williamson 1980; Asaeda et al. 2001). The hunger level of a copepod may increase the feeding current speed and decrease the duration for completing several or all steps of the predation sequence. Other factors that influence predation interactions between copepods and their prey are age, size,

sex, and reproductive stage of the predator (Yen 1983; Kumar 2003; Dhanker et al. 2012). Prey ingestion rates were inversely proportional to gut fullness of the predator as shown in the present study. A previous study showed that *P. annandalei* became more selective when feeding on rotifer prey following a certain duration of feeding (Dhanker et al. 2012). In contrast, the hunger level is an important promoter of prey ingestion; animals do not show feeding stimuli or become more selective when satiated (Croy and Hughes 1991; Salvanes and Hart 1998). Starved calanoids are more active in generating feeding currents compared to satiated individuals. The gut content affects prey ingestion probabilities and handling times in *P. annandalei* (Dhanker et al. 2012). Prey ingestion rates and gut fullness generally show an inverse relationship (Croy and Hughes 1991). In this study, *P. annandalei* at near satiation showed a tendency to reject ciliates following prey entrainment in the feeding appendages. Conversely, the rejection of an entrained cell was minimal when copepods were at their maximum hunger stage, which may be attributed to the number of ciliate cells in the gut. When satiated, *P. annandalei* did not show active swimming towards prey and frequently rejected prey entrained in its feeding appendages. Price et al. (1983) reported that calanoid copepods used their second maxillae for the reverse motion of rejecting a prey following capture. Prey rejection by copepods may be due to gut fullness, prey size, unpalatability of the prey, and longer handling times (Williamson 1980, 1987).

Previous studies showed that copepods initially entrain their prey in a feeding current and attack the prey after detection (Svensen and Kiørboe 2000, Jakobsen et al. 2005). The feeding current beats the bands of the microzooplankton prey (Conover 1981). We observed that *P. annandalei* began moving slowly towards a ciliate until it was in close proximity and then used feeding currents to capture the prey in its feeding appendages. Different feeding modes in *P. annandalei* may be attributed to the differential size and mobility of ciliates compared to rotifers.

The prey searching time of organisms depends on the hunger level of the predator (Williamson 1980) and other factors such as swimming speed, and size and abundance of prey and predators (Mazzocchi and Paffenhöfer 1999; Rao and Kumar 2002; Uttieri et al. 2008; Wu et al. 2010; Mahjoub et al. 2011; Kumar et al. 2012). In the present study, gut fullness did not have a significant effect on handling times or prey detection in female *P. annandalei*, but males were affected by hunger levels. Searching and handling times were higher in male *P. annandalei* with increased hunger levels than in females. We discovered that starved calanoids were more active in searching for ciliates compared to satiated ones. This study concluded that prey detection by *P. annandalei* is influenced by the hunger level, sex, and distance between the prey and predator.

Several studies demonstrated that the hunger state of copepods has a significant effect on ciliate consumption (Runge 1980; Jonsson and Tiselius 1990). Runge (1980) demonstrated that grazing rates of starved *Calanus pacificus* increased 1.5 ~ 3-fold compared to well-fed copepods. In our earlier study (Dhanker et al. 2012), prey handling times directly affected rotifer ingestion probabilities in *P. annandalei* with increasing hunger levels in the predator. Ciliate ingestion probabilities showed a direct relationship with the hunger level of *P. annandalei* regardless of sex, which is a similar result as in the present study. A minimum number of ciliate cells were rejected at the highest hunger level in male and female *P. annandalei*.

Conclusions

The perennial abundance in natural habitats and efficiency of utilizing food from autotrophic and heterotrophic food suggest that feeding habits of *P. annandalei* are highly adaptive and it can derive nutrients during periods of low primary production. Moreover, single algal species did not alter ciliate ingestion rates of *P. annandalei*, which reflects its adaptive nature of feeding. Copepods are unable to synthesize HUFAs, which are essential for their growth and reproduction. Phytoplankton feeders must obtain these essential HUFAs in their diet (Støttrup and Jensen 1990). *T. chui* is a moderate source of HUFAs, and *Isochrysis* is rich (9% total fatty acids) in DHA but has scant EPA (Dunstan et al. 1993). Therefore, either alga is not nutritionally sufficient for the copepod, but a combination of the two species is suitable. This limits the chance of nutritional deficiencies during copepod development and reduces the risk of food shortages that may occur from the failure of algal culture. The present study points to the role of *P. annandalei* in forming a link between the microbial loop and classical food chain, which expedites the flow of bacterial carbon to higher trophic levels in estuarine ecosystems (e.g., the Danshui estuary; Hwang et al. 2010). Laboratory experiments such as those in this study are important for estimating ingestion rates at specific prey concentrations and determining what factors influence those rates. However, better estimates of natural microzooplankton concentrations and the size and permanence of patches are necessary before such studies can be used to quantitatively measure mortality from predation in nature.

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