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## Nymphal Growth, Life Cycle, and Feeding Habits of *Potamanthus luteus* (Linnaeus, 1767) (Insecta: Ephemeroptera) in the Bormida River, Northwestern Italy

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**Stefano Fenoglio, Tiziano Bo, José Manuel Tierno de Figueroa, and Marco Cucco (2008)** Nymphal growth, life cycle, and feeding habits of *Potamanthus luteus* (Linnaeus, 1767) (Insecta: Ephemeroptera) in the Bormida River, northwestern Italy. *Zoological Studies* 47(2): 185-190. *Potamanthus luteus* (Linnaeus, 1767), the only representative of the family Potamanthidae in Europe, exhibits explosive growth in terms of its numerical presence and mass increase in springtime. The aim of this study was to analyze the pattern of the size increase, the life cycle, and the feeding habits of *P. luteus* nymphs in the Bormida River of northwestern Italy. In particular, we investigated the growth patterns of 3 populations, located at stations at different elevations. Thermal regimes of the 3 stations were assessed using a HoBo WaterPro Datalogger. In 2006, immature stages of *P. luteus* were collected from the time they appeared in Mar. until their emergence as adults in June. In total, 1407 nymphs of *P. luteus* were collected and measured. Water temperatures differed between the 3 stations, and nymphs at the lowest, warmer station developed faster and emerged before nymphs at the upper stations. This species shows a rapid univoltine seasonal life cycle. The diets of the nymphs at the 3 stations were similar, being mainly based on fine detritus. We suppose that higher temperatures strongly influenced bioenergetics and development and played a major role in fostering faster growth in the downstream population of this mayfly. <http://zoolstud.sinica.edu.tw/Journals/47.2/185.pdf>

**Key words:** Mayfly, Potamanthidae, Nymphal biology, Apennines, Southern Europe.

The Potamanthidae is a small, relatively uncommon mayfly family, belonging to the Ephemeroidea group. Potamanthidae nymphs are clingers or sprawlers, usually living in areas with a coarse substratum (Edmunds 1984). Generally, members of the Potamanthidae are considered collector-gatherers, feeding mainly on detritus (Cummins and Merritt 1996, Tachet et al. 2002, Monakov 2003). The genus *Potamanthus* Pictet has a Holarctic distribution (Edmunds et al. 1976), and *Potamanthus luteus* (Linnaeus, 1767) is the only representative of this family in Europe. Nymphs of this species have a sub-cylindrical body, both sides of the cerci and paracercus are fringed, and the gills are laterally oriented, with the 1st pair reduced and the other pairs double and elongate

with fringed margins.

This species is considered quite rare but locally abundant, with a fragmentary and disconnected distribution. In Italy, it was originally reported from the Northern Apennine, and is actually known only from the northern peninsula (Belfiore 1994). In a recent work, investigating the benthic fauna of 424 stations belonging to 129 streams and rivers in Piemonte, northwestern Italy, *P. luteus* was found at only 22 stations: in this area, *P. luteus* seems to prefer medium and large rivers, with pluvial regimes and warmer temperatures, avoiding alpine-born lotic systems (Regione Piemonte 2000).

*Potamanthus luteus* is considered an univoltine species, with a characteristic life cycle

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(Torralba Burial and Ocharan 2005). Adults are short-lived (6-9 h for the preimago and 24-48 h for the imago), mainly nocturnal flyers, occurring in Europe in June-Aug. (Grandi 1960, Landa 1968, Messori and Tosi 2003). In the northern Italian Apennines, nymphs are present in streams for only a very short period, generally a few months from late springtime to early summer (pers. obs.). In this phase, nymphs show an explosive growth, with an impressive size increase.

The aims of this study were to analyze the growth (i.e., the size increase) and provide new data on the life cycle and feeding habits of *P. luteus* nymphs in the Bormida River, a typical Apennine lotic system. In particular, we investigated the growth of 3 populations, located at stations on the Bormida River sited at different elevations; we hypothesized that differences in thermal regimes would be reflected in differences in postembryonic development.

## MATERIALS AND METHODS

The study was done in the Bormida River, a typical Apenninic river, with a pluvial regime and a source at 700 m in elevation. Samplings were made at 3 stations (Fig. 1): Gorzegno (323 m; 44°30'37"N, 8°08'03"E), Cortemilia (238 m; 44°36'04"N, 8°11'52"E), and Monastero Bormida (177 m; 44°38'59"N, 8°19'14"E).

Thermal regimes of the 3 sites were assessed using a HoBo WaterPro Datalogger. In order to carefully follow temperature changes throughout the study, a single datalogger was anchored in the substratum at each station, and a temperature value was collected every 10 min. Major abiotic parameters were measured on each sampling date with Eijkelkamp 13.14 and 18.28 portable instruments, and are reported in table 1.

The sites were inspected at least monthly in the period 5 Jan. 2006-14 Feb. 2007, and

immature stages of *P. luteus* were collected from the time they appeared (Mar. 2006) until their emergence as adults (June 2006). At the 3 sampled sites, nymphs were collected on 10 dates (28 Mar., 7, 13, 20, and 29 Apr., 9, 16, 23, and 31 May, and 21 June 2006) with a 250  $\mu\text{m}$ -mesh kick-net, and on each occasion, large numbers of individuals were found. From these samples, we generally selected at least 50 individuals/date/site in a random way. The nymphs were stored in 75% ethanol and later measured in the laboratory with an ocular micrometer mounted on a Nikon SMZ1500 stereomicroscope (to an accuracy of 0.01 mm). The following 3 measures were taken from each individual: a) pronotum width, b) femur length, and c) total length (excluding the cerci). These data were also employed to generate life cycles graphs.

Furthermore, the gut contents of 30 nymphs (10 for each station, representing all different sizes) were analyzed following the methodology of Bello and Cabrera (1999). Each nymph was introduced to a vial with Herwitgs' liquid for 24 h and then placed in an oven at 65°C, to clarify tissues and to allow gut content analysis. Afterwards, cleared individuals were positioned individually on a slide glass with a cover glass, and they were inspected at 40-90x magnification with an Olympus microscope. The different components of the gut contents were differentiated in the following categories: fine particulate organic matter (FPOM), algae, sand, and animals. We quantified the percentages these 4 categories occupied in each gut.

We used an analysis of covariance (ANCOVA) statistic to test for significant differences in temperature and size among the 3 stations. In the ANCOVA, temperature and size were used as the response variables, the sampling date as the covariate, and the station as the independent categorical variable. Differences in gut contents of nymphs collected at the 3 sampling sites were

**Table 1.** Abiotic characteristics of sampling stations (mean  $\pm$  SD,  $n = 10$ , except for river depth and width)

Sampling station	Gorzegno	Cortemilia	Monastero
Velocity (m/s)	195.8 $\pm$ 70.6	169.6 $\pm$ 84.3	218.0 $\pm$ 40.2
Conductivity ( $\mu\text{S}/\text{cm}$ )	468.6 $\pm$ 127.4	462.3 $\pm$ 124.9	523.6 $\pm$ 55.7
pH	7.9 $\pm$ 0.11	7.9 $\pm$ 0.09	7.9 $\pm$ 0.11
O <sub>2</sub> (mg/l)	5.4 $\pm$ 3.65	6.2 $\pm$ 3.17	5.4 $\pm$ 3.16
Maximum depth (cm)	30.3	36.2	35.0
Maximum width (m)	10.9	17.3	19.4

examined by analysis of variance (ANOVA), with FPOM and algal abundance as dependent variables, and stations as categorical variables. Percentage values were arcsine-transformed before the analyses. Statistical analyses were performed with Systat 8.0 (Wilkinson 2000) and Statistica 7.1 (StatSoft 2005).

## RESULTS

In total, 1407 nymphs of *P. luteus* were

collected and measured: 459 from Gorzegno, 498 from Cortemilia, and 450 from Monastero Bormida.

### Size increase

Analyzing the relationships among the 3 morphometric parameters measured, we detected a significant correlation between total length and pronotum width (Pearson correlation test = 0.97,  $p < 0.001$ , Fig. 2), total length and femur length (Pearson correlation test = 0.96,  $p < 0.001$ , Fig. 3), and femur and pronotum measurements (Pearson

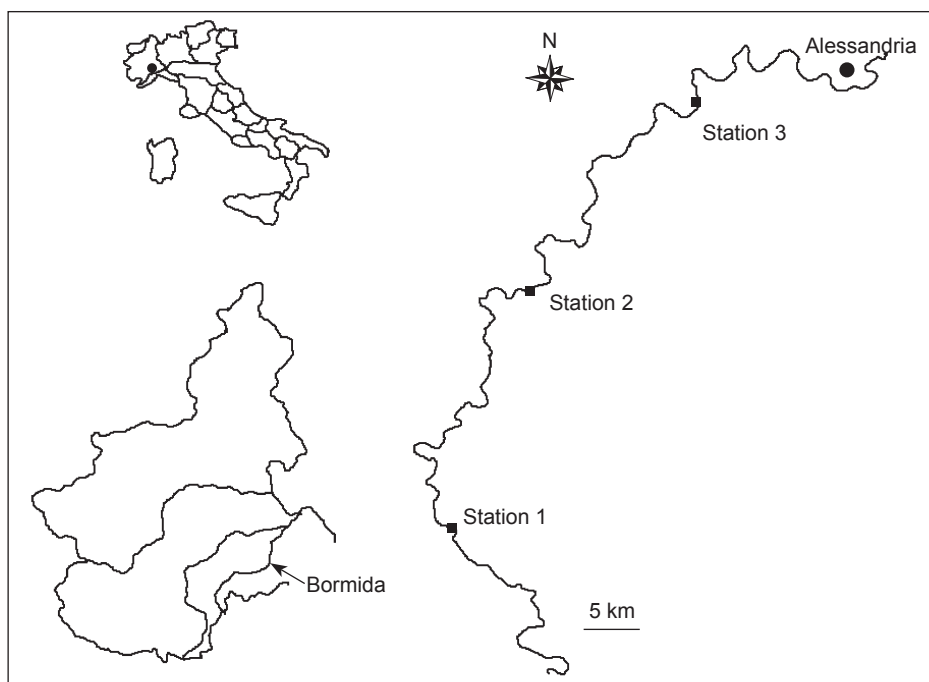


Fig. 1. Bormida River and sampling stations.

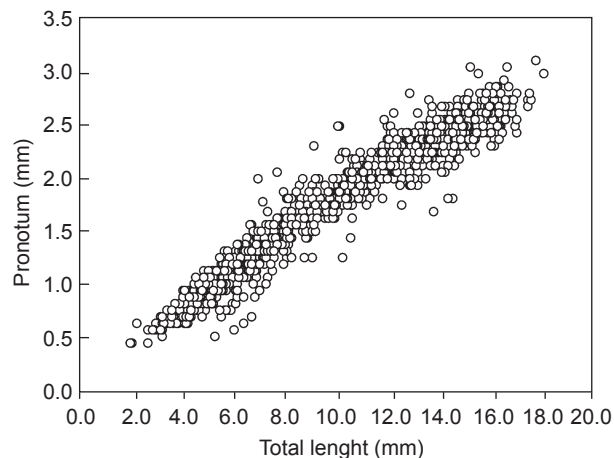


Fig. 2. Relationship between total length and pronotum width of *Potamanthus luteus* nymphs.

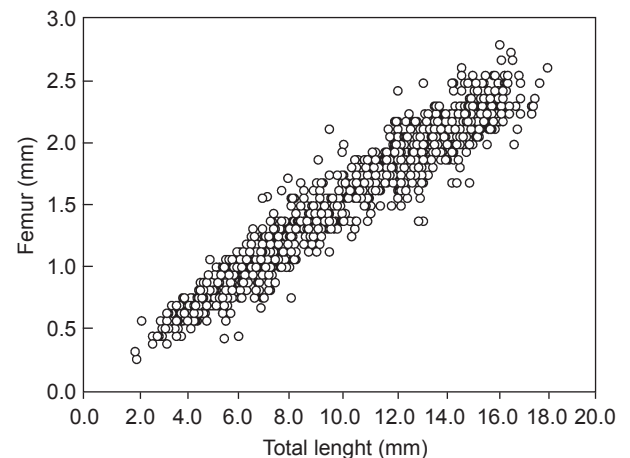


Fig. 3. Relationship between total length and femur length of *Potamanthus luteus* nymphs.

correlation test = 0.97,  $p < 0.001$ ). For this reason, in the following analyses, we only employed total length as a concise indicator parameter of growth.

We detected obvious differences in the thermal regimes among the 3 stations. The daily mean temperatures significantly differed (effect of date: ANCOVA  $F_{1,512} = 744.8$ ,  $p < 0.001$ ; difference between sites:  $F_{2,512} = 33.9$ ,  $p < 0.001$ ). Temperatures showed an evident increase moving downstream: the mean  $\pm$  SD temperature at Gorzegno was  $17.3 \pm 2.4^\circ\text{C}$ , at Cortemilia  $17.5 \pm 2.5^\circ\text{C}$ , and at Monastero  $18.7 \pm 2.7^\circ\text{C}$ . The degree days were 2971 at station (stn.) 1, 2998 at stn. 2, and 3200 at stn. 3.

We detected a significant difference in the growth pattern of total length of *P. luteus* nymphs (effect of date: ANCOVA  $F_{1,1276} = 3205.7$ ,  $p < 0.001$ ; difference among sites:  $F_{2,1276} = 9.74$ ,  $p < 0.001$ , Fig. 4), femur length (effect of date: ANCOVA  $F_{1,1276} = 2797.9$ ,  $p < 0.001$ ; difference among sites:  $F_{2,1276} = 12.6$ ,  $p < 0.001$ ), and pronotum width (effect of date: ANCOVA  $F_{1,1276} = 2556.4$ ,  $p < 0.001$ ; difference among sites:  $F_{2,1276} = 7.54$ ,  $p < 0.001$ ). Maximum body lengths were in the range previously reported for this species (Obdrlik et al. 1979).

**Life cycle**

The life cycle of *P. luteus* is univoltine, and was similar at the 3 studied stations (Figs. 4-6). In our study area, nymphal growth extended over 3 mo only. Imagoes were found from mid-June. No nymphs were found in the river during the period of 5 Jan.-28 Mar. 2006 or 21 June 2006-14 Feb.

2007. Thus, *P. luteus* exhibited a rapid seasonal life cycle sensu Hynes (1970) in our study area.

**Diet**

Gut contents of nymphs were mostly composed of FPOM (found in 90.0% of nymphs, comprising 56.3% of the gut contents) and algae (found in 23.3% of nymphs, comprising 8.0% of the gut contents). Invertebrates (small Chironomidae larvae) were only found in 2 guts. Interestingly, we found sand in 70% of the examined guts. There were no differences in gut contents among the 3 stations, considering both the percentage of FPOM (ANOVA  $F_{2,24} = 0.15$ ,  $p = 0.86$ ) and the percentage

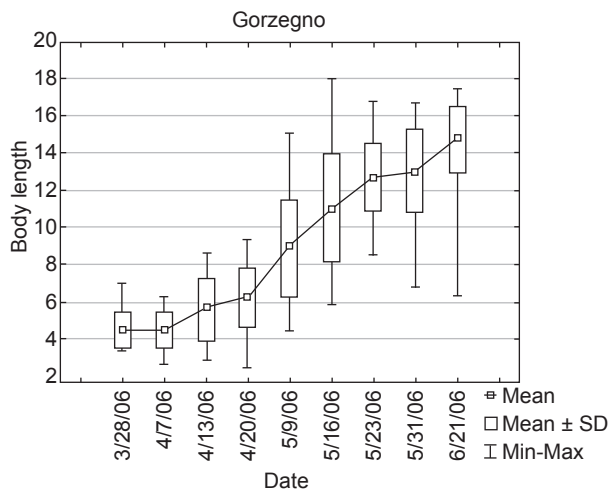


Fig. 4. Life cycle of *Potamanthus luteus* at the Gorzegno station.

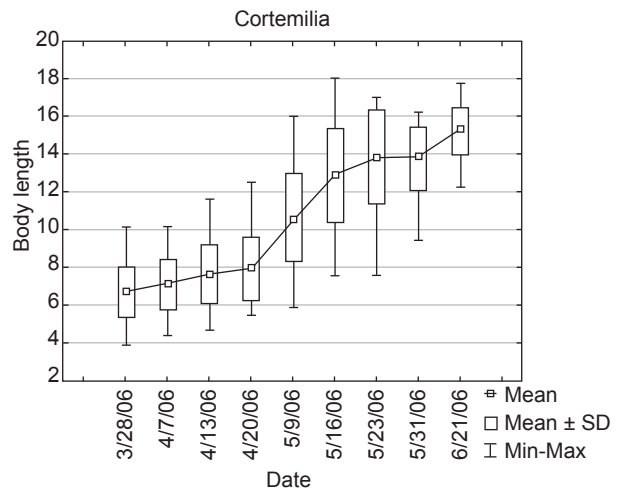


Fig. 5. Life cycle of *Potamanthus luteus* at the Cortemilia station.

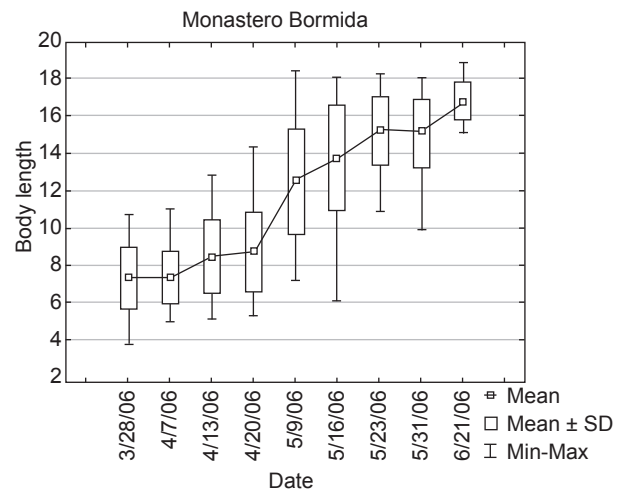


Fig. 6. Life cycle of *Potamanthus luteus* at the Monastero Bormida station.

of algae (ANOVA  $F_{2,24} = 0,61, p = 0.55$ ).

## DISCUSSION

In flowing freshwater systems, some invertebrate species show explosive growth (Peràn et al. 1999). For example, *Oligoneuriella rhenana* (Imhoff, 1852) nymphs (Ephemeroptera: Oligoneuriidae) are present in northwestern Italian streams for only a very short period (generally from late Apr. to early summer): during these few months, nymphs show explosive growth with impressive size increases (Fenoglio et al. 2005b). Also stoneflies of the genus *Brachyptera* Newport, 1849 in Apenninic and subalpine streams are present as nymphs for a very short period and also exhibit impressive growth (Agosta et al. 2001).

We detected the same pattern in our studied species, *P. luteus*, which showed a sudden increase in length during spring. In Mar., nymphs appeared in the benthic community, but they were small, reaching a mean total length of about 5 mm. After 3 mo, the mean total length had tripled to a mean of about 14 mm.

*Potamanthus luteus* in our study area has a shorter and more-intense growth period than other *Potamanthus* species, such as *P. myops* (Walsh, 1863). Munn and King (1987a b) reported that *P. myops* nymphs appeared in central Michigan streams in Oct., then they ceased growth during winter months, and finally showed explosive growth in spring. *Potamanthus luteus* nymphs in Piemonte usually appeared at the end of winter or early spring and showed a more-concentrated growth period.

Among the different parameters that can influence growth in aquatic insects, temperature, nutrition, and photoperiod seem to play key roles (Ward 1992). Comparing the size increases of the 3 populations in the Bormida River, we detected significant differences, with the population of the lowest and warmest station growing more rapidly than the 2 upstream populations.

*Potamanthus luteus* exhibits a univoltine life cycle in the studied river, as in other parts of its distribution area (Macan 1979, Torralba Burrial and Ocharán 2005) which coincides with what is generally accepted for different species of the genus *Potamanthus* (Munn and King 1987a). Because adults fly in June, eggs are probably soon deposited in mid-summer. However, it is not clear whether or not this species spends the remaining months in the egg stage (in a dormant stage), or

as very small nymphs with very low growth, as has been reported for other ephemeropteran species (Brittain 1982). In the latter case, the absence of nymphs during all of autumn and winter can only be explained by the use of a hyporheic habitat. Nevertheless, more studies are needed to obtain well-supported conclusions.

According to the literature, nymphs of *P. luteus* predominantly feed on macrophyte tissue and detritus, and its herbivorous nature was confirmed by food-choice experiments (Monakov 2003). Regarding its functional feeding group sensu Cummins and Merritt (1996), this species, as other members of the Potamanthidae, can be classified as mainly a collector-gatherer (Edmund 1984). Our findings confirm these data, supporting a role as a collector-gatherer for this species. The presence of some small Chironomidae residues in the gut content may likely be a consequence of incidental ingestion more than active predation. At the studied stations, leaves (present as coarse particulate organic matter, CPOM) are actively fragmented during the winter by fungi, bacteria, and macroinvertebrates belonging to the functional trophic group of shredders (Fenoglio et al. 2005a). By the spring, a considerable part of the CPOM has been transformed into FPOM. This FPOM is consumed by other organisms, such as *P. luteus* nymphs. Thus, this species takes advantage of the high availability of food in spring for rapid growth, and it can fly before the summer period, when the aquatic environment is less favorable due to low hydrological levels and warm, less-oxygenated waters.

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