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Host Relation, Size and Reproduction in the Burrowing Barnacle

*Trypetesa lampas* (Hancock) (Crustacea Cirripedia Acrothoracica)

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**Sofie K. D. Nielsen, Jens T. Høeg, and Yoichi Yusa (2016)** The aim of this study is to investigate the population biology of the burrowing barnacle *Trypetesa lampas*, a symbiont of hermit crabs and representing the little known cirripede group Acrothoracica. We put special emphasis on the host-barnacle relation and reproduction. A total of 341 hermit crabs from the west coast of Sweden was captured in November 2009 and August 2010, and examined for the associated burrowing barnacles. We found a mean load of 1.4 *T. lampas* per host and an average prevalence of 31.4% with no seasonal variation. Male hermit crabs also carried *T. lampas*, indicating that *T. lampas* does not rely on egg-predation to any substantial degree. The *T. lampas* load was positively related to host size, but otherwise their frequency distribution did not differ from random. The position of the burrow in the columella of the shell was positively associated with *T. lampas* size. Reproduction seems to occur throughout the year. We found ovigerous females also in winter, although less frequently than in summer, and no difference in the number of dwarf males between the summer and winter samples. The data from the present study site deviates in many respects (prevalence, female and male load, reproductive cycle, host relation) from previous studies on this and closely related acrothoracican species. This emphasizes that a basic lack of knowledge still exists concerning most aspects of acrothoracican reproduction, life cycles and host relation.

**Key words:** Acrothoracica, Barnacle, Host-parasite relation, Reproductive ecology, *Trypetesa lampas*.

**BACKGROUND**

Compared to the pedunculated and acorn barnacles (Thoracica) and the parasitic barnacles (Rhizocephala), the burrowing barnacles (Acrothoracica) have for long remained the by far least studied group of cirripeds, even though they were among the first that attracted the attention of Darwin (Darwin 1851, 1854; Stott 2003). Turquier (1972 and citations therein) offered an excellent but unfortunately little cited account. Recently there have been significant advances in acrothoracican systematics, phylogeny and larval morphology (Kolbasov 2009; Kolbasov and Høeg 2007; Kolbasov et al. 2014), but studies on the ecology of this interesting group of barnacles remain very few (Kühnert 1934; Williams et al. 2010, 2011; Chan et al. 2014a).

The Acrothoracica resembles the Thoracica in having terminal cirri for food collection, but they are otherwise highly modified (Anderson 1994; Kolbasov 2009; Chan et al. 2014b). They lack any calcareous shell plates and are mostly symbionts that excavate burrows in calcareous substrata in the marine environment including, molluscan and thoracican shells, corals, bryozoans and calcareous rocks (Tomlinson 1969, 1987; Chan et al. 2014b). Feeding of Acrothoracicans are divided into two types, with the majority being suspension feeders and only *Trypetesa* having modified cirri, for putatively feeding on food debris in hermit crab shells. Most acrothoracican species

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are epizoic (e.g. on corals), and those of the family Trypetesidae are symbionts, occupying burrows inside gastropod shells inhabited by hermit crabs (Fig. 1). Like all rhizocephalans and some thoracican barnacles, the Acrothoracica have a dioecious sexual system with large feeding females associated with non-feeding dwarf males (Høeg 1995). The female acrothoracican has

Fig. 1. The morphology of Trypetesa lampas. (A) The burrow opening inside the gastropod shell. (B) Schematic view of the burrow seen from inside the shell. (C) Adult specimen exposed by cracking the burrow open, the body consists of a basal disc housing the ovary and a “capitulum” with the mantle cavity and soma (= mouth cone and thorax with cirri), a small mass of embryos is brooded in the mantle cavity of this specimen. (D) Small specimen in situ in the burrow but partially exposed; before dislocation the mantle aperture (see E) would have faced the burrow opening. (E) Same specimen as in D, but from another angle showing a single dwarf male in rectangle area. (F) Magnification of rectangle in E; the dwarf male body consists of a bulbous basal part with attachment processes partially embedded in the female mantle and an elongated part traversed by a narrow “penis canal” (see inset). (G) Adult specimen removed from burrow; the basal disc is attached to the bottom of the burrow; the dotted line shows how the length of the “capitulum” is measured. (H) Schematic drawing showing the position of the dwarf males. (I) Two dwarf males and an un-metamorphosed male cyprid attached close together where indicated in H; note the large pigment bodies, representing degenerated compound cypris eyes in the basal body part of the males. The line drawings are modified from various sources.
a brood chamber (mantle cavity) that connects with the exterior through a slit-like opening in the burrow (Figs. 1A, B). Inside the burrow, the minute males are attached either externally on the female body (Figs. 1H, I) or (Berndtia) to the walls of the burrow, but many details concerning their reproductive biology remain obscure (Gotelli and Spivey 1992).

Despite being highly specialized and with an interesting symbiotic mode of life, few papers have focussed on the population biology and relationship to the hosts in acrothoracican barnacles, including those associated with hermit crabs (e.g., Kühnert 1934; Uchinomi 1964; Turquier 1972; Williams et al. 2011). Here we study the acrothoracican Trypetesa lampas (Hancock, 1849) that, in a locality on the west coast of Sweden, has a high prevalence in the hermit crab Pagurus bernhardus (Linnaeus, 1758). We have sampled this population at two different seasons to test the following hypotheses (H) concerning this barnacle and its relation to the host. First, T. lampas can live for several years (Kühnert 1934), and we therefore (H1) predict only small seasonal variation in prevalence. Next, the dioecious sexual system means that there is no reproductive need for gregarious settlement, and we accordingly (H2) expect a random frequency distribution of T. lampas females in the host shells. But larger shells should (H3) offer more substrate for settlement, whence we predict a correlation between crab/shell size and number of associated T. lampas. Based on Williams et al. (2011) we wanted to test the hypothesis that T. lampas is primarily an egg predator that (H4) prefers female crabs and (H5) specifically those that carry eggs. We (H6) expect a seasonal variation in reproduction, since our study site is in temperate waters, where most other barnacles, including the parasitic ones, show such a pattern (Nilsson-Cantell 1978; Høeg et al. 1989; Høeg and Lützen 1985). T. lampas can carry multiple males per female, but broods are very small (SKDN and JTH personal observation), so following Gotelli and Spivey (1992) we (H7) predict that sperm competition will lead to a non-random distribution of males on the females.

MATERIALS AND METHODS

Locality and Sampling

The hermit crabs were collected in November (winter) 2009 and August (summer) 2010 near the Kristineberg Marin Biological Station, Sven Lovén Centre, situated at the west coast of Sweden (58°16'N; 11°25'E). Both winter and summer samples were collected from Bökeviken, a small protected inlet ca. 1 km across and situated near the opening of the Gulmar Fjord; depth ≈ 20 m. The location was chosen because hermit crabs (Pagurus bernhardus) are numerous and have a high prevalence of Trypetesa lampas. The P. bernhardus were dredged from the gravel bottom using an Agassiz-trawl and brought back alive to the laboratory. A total of 341 P. bernhardus were collected, and the samples did not contain other species of hermit crabs, although both Anapagurus chiroacanthus (Liljeborg, 1856) and Pagurus cuanensis (Bell, 1846) are frequent nearby in slightly deeper water localities. The majority of the shells (86%) originated from the snail species Littorina littorea (Linnaeus, 1758) and somewhat fewer (14%) from Hinia (syn. Nassarius) reticulata (Linnaeus, 1758). The very few empty gastropod shells in the samples were not counted or screened for T. lampas.

Specimen examination: The hermit crabs were removed by gently cracking their shells by means of a clamp. The shell pieces were examined under a dissection microscope for T. lampas females, which often have a characteristic pink-orange color in vivo (Figs. 1C-E, G). For the hermit crabs we recorded: sex, carapace length and although their burrows were often partially damaged we could normally keep them alive in flowing seawater for at least 2-3 days. All T. lampas specimens were eventually preserved in 1% formalin in seawater. In female T. lampas the body is divided into a basal disc containing the ovary and a “capitalum”, containing the mantle cavity, in which is suspended the “soma” carrying the mouth cone and the cirri (Fig. 1G). We recorded the number of live female specimens per shell, their size (as the length of the “capitalum”, also called “opening bud”, see Fig. 1G), the number of dwarf males per female and whether the females had brooded embryos or larvae in their mantle cavity. Some females clearly had mature eggs in the ovary, and this condition was also recorded (see Additional File 1 for recorded data). Selected T. lampas were photographed in vivo in a dissection microscope with a Q-imaging Evolution or Olympus
C5050 digital camera.

**Statistical Analyses**

To assess the effects of environmental factors on various population traits of *T. lampas*, GLMs (generalised linear models) with model selection procedure were used. In the GLMs, log link and Poisson error functions were used for the count response variable (e.g., number of female *T. lampas* per crab). Likewise, logit link and binomial error functions were used for binomial data (e.g., whether female *T. lampas* were located in the columnella or outer side of the shell), and identity link and normal error functions for continuous data (e.g., size of each female *T. lampas*). In the model selection procedure, the model with the lowest AICc (corrected Akaike’s information criteria; Burnham and Anderson 2004) value was selected as the best model.

The explanatory variables considered in all the initial models were host size, host sex, and season, except for the analysis of the number of *T. lampas* per female host, where the presence/absence of host eggs was included instead of host sex. In addition to these, the presence/absence of dwarf males was included in the analysis of maturation (evidenced from having eggs in either gonad or mantle cavity) of female *T. lampas*, and the presence of eggs laid in the mantle cavity was included in the analysis of the number of dwarf males per female *T. lampas*. Although we did not record shell size for each host crabs, shell size and host size were positively correlated (r = 0.40; P < 0.01) (Fowler et al. 2006 p. 239).

**RESULTS**

**Prevalence, Load and Frequency Distribution of *T. lampas***

The mean prevalence of *T. lampas* in the hermit crab population (= the proportion of crabs with *T. lampas*) was 31.4% (98 of 312 crabs), and it did not differ between winter and summer (November 31.2%; August 32.7%). The mean load (= the number of *T. lampas* females per crab) was 1.4 for all crabs with *T. lampas* (181 females on 127 crabs); the November samples had a mean load of 1.3 and the August samples had 1.6 (Table 1). The frequency distribution of the load (Fig. 2) differed significantly from a random one (χ² = 98.94, P < 0.001; Pearson χ² goodness of fit test), with a greater number of hosts without load. This indicates that some explanatory factor(s) is needed to explain the frequency distribution. The sex ratio in the total crab population was strongly female biased (72.0% females and 28.0% males among 143 crabs determined to sex). In contrast, the *T. lampas* carrying crabs comprised more males (35.5% males among 67 crabs determined to sex).

Among the explanatory variables initially considered (i.e., host size, host sex and season), both host size and host sex were selected in the final model after the model selection procedure. The number of *T. lampas* increased with body size of the host (N = 98, likelihood χ² = 4.76, P = 0.03). In addition, male crabs had a greater number than female crabs (likelihood χ² = 7.84, P = 0.005). Season was not selected in the final model, indicating that the difference between seasons was not significant. The residual deviance of the final model (94.71) divided by the residual degrees of freedom (95) was nearly 1, indicating that the residual after incorporating the effects of size and sex of the host did not differ from random (Poisson) distribution.

In contrast, neither host size, presence of eggs or season remained in the final model explaining the number of *T. lampas* per female host. This indicates that neither of these factors, including the presence of eggs, affected the load in the female hermit crabs.

**Size and Reproduction of *T. lampas***

*Females*: To assess the possible factors explaining the size variation of female *T. lampas*, host size, host sex, load, position and season were included in the initial model. Among them, only the position was selected in the final model; *T. lampas* females situated on the columnella were significantly larger than those in the peripheral wall parts of the shell (N = 111, likelihood χ² = 6.06, P = 0.01).

Mature *T. lampas* females had eggs either in the ovary (unlaid) or in the mantle cavity (laid and usually fertilised) (Table 2). Thus, factors possibly affecting female maturity were analysed including host size, host sex, *T. lampas* size, season and presence of males as explanatory variables in the initial model. The final model included the size of *T. lampas* and the presence of males. As expected, larger *T. lampas* females were more likely to have eggs (N = 117, likelihood χ² = 14.41, P < 0.001). Moreover, females with males were more likely to have eggs than females lacking a male (likelihood
\( \chi^2 = 6.41, P = 0.01 \).

The minimum size (capitulum length, see Fig. 1G) at which females carried eggs in the mantle cavity was 1.40 mm. Then among females with a capitulum length of 1.40 mm or larger, factors responsible for brooding were analysed using the same explanatory variables. As a result, both size of \( T. \) lampas and season affected the brooding: larger females were more likely to be ovigerous than smaller ones (\( N = 110 \), likelihood \( \chi^2 = 7.08, P = 0.008 \)) and in summer than in winter (likelihood \( \chi^2 = 16.20, P < 0.001 \)).

\textit{Males}: The mean number of males per female was 0.63 (= 75/120), with only 35.1% of the females carrying a male (Table 3). The frequency distribution of males per female (Fig. 3) differed significantly from a random one (\( \chi^2 = 42.49, P < 0.001 \); Pearson \( \chi^2 \) goodness of fit test); there were

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
Collection & November 2009 & August 2010 & Total \\
\hline
Hermit host examined & 263 & 78 (49)\(^1\) & 341 (312)\(^1\) \\
\( T. \) lampas associated crabs & 82 & 45 (16)\(^1\) & 127 (98)\(^1\) \\
Prevalence & 31.2\% (82 of 263) & 32.7\% (16 of 49) & 31.4\% (98 of 312) \\
\( T. \) lampas total # & 110 & 71 & 181 \\
Load (mean \( T. \) lampas # / host) & 1.3 (110 on 82) & 1.6 (71 on 45) & 1.4 (181 on 127) \\
\hline
\end{tabular}
\caption{Prevalence and load of the burrowing barnacle \( T. \) lampas in gastropod shells inhabited by the hermit crab \( P. \) bernhardus.}\label{tab:1}
\end{table}

\(^1\) Not all non-host crabs were recorded in the August sample; the numbers in parenthesis are those used for the prevalence statistics.

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
Collection & November 2009 & Summer 2010 & Total \\
\hline
\( T. \) lampas investigated for eggs & 62 & 55 & 117 \\
\( T. \) lampas with eggs & 35 (56.6\%) & 33 (60.0\%) & 68 (58.1\%) \\
With undeveloped eggs & 22 (62.9\%) & 4 (12.1\%) & 26 (38.2\%) \\
With developed eggs/embryos & 13 (37.1\%) & 29 (87.9\%) & 42 (61.8\%) \\
\hline
\end{tabular}
\caption{\( T. \) lampas reproduction: females with egg/embryos.}\label{tab:2}
\end{table}

Reproductive data on \( T. \) lampas. The number and percentage of \( T. \) lampas with either developing eggs or mature eggs/embryos.
more females without a male than expected by chance. Thus, the effects of factors (host size, host sex, size of *T. lampas* female, female maturity and season) were studied on the number of males per female. Among them, only size of females was selected in the final mode (N = 117, likelihood $\chi^2 = 15.10, P < 0.001$). Some of the males from the November sample looked very transparent, but without histology it was not possible to decide with certainty whether they were capable of yielding sperm or not. Season was not selected as an explanatory variable in the final model.

**DISCUSSION**

**Prevalence, load and frequency distribution of *Trypetesa lampas***

The high prevalence of *T. lampas* found at our study site, did not vary significantly with season, confirming our hypothesis (H1) that this, rather long-lived species, show small but seasonal population fluctuations. The mean prevalence of 31.4% is in accord with a previous unpublished survey from August 1991, where 30-40% prevalence was observed (Høeg unpublished observations). Thus, the prevalence might be rather constant over years at this locality. Unlike most acrothoracicans (Kolbasov et al. 2014, Martin et al. 2014), *T. lampas* have a number of free-swimming naupliar stages (Kühnert 1934), and development from release to the cypris settlement stage lasts about 1 week at local temperatures (Høeg unpublished observations). This offers some capacity for dispersal, and the apparently constant prevalence, probably reflects the conditions in the local habitat rather than a localized recruitment. Hermit crabs seem to migrate very little and this would further enhance such local effects (Stachowitch 1979).

The few available studies on *T. lampas* and other species of this genus, demonstrate that extensive variation prevails across local populations, in both prevalence and load. For instance, the prevalence of *T. lampas* was only...
4.2% in the eastern Mediterranean coast of Spain (Williams et al. 2011), 24% in Florida, North Eastern Gulf of Mexico (Gotelli and Spivey 1992) and 67% in Anglesey, Wales (White 1969). Turquier (1972), studying the closely related Trypetesa nassaroides, reported a yearly variation from 25 to 45% in Brittany, France, and in the Philippines Williams and Boyko (2006) found a 3% prevalence of Tomlinsonia mclaughlinea.

Contradicting our hypothesis (H2), the frequency distribution of T. lampas in P. bernhardus differed significantly from random, with more hosts having no T. lampas than expected. However, after incorporating the effects of host size and sex, the residual deviance did not differ from random distribution. This suggests the absence of both gregarious settlement and intraspecific competition of T. lampas females. Gregarious settlement is a common feature in barnacles (Walker 1995), but mostly driven by the need to sit within copulation distance of a partner (Spremberg et al. 2012). With a dioecious sexual system with dwarf males as in T. lampas, gregariousness of females incurs no advantage for reproduction. Competition among commensals or parasites is common, but should have been low in this case, considering the low load (1.4 T. lampas per host). We might expect a more severe competition, when load is higher. In fact, Gotelli and Spivey (1992) reported a much higher load (30.1) for the same species, the maximum being 114. But it should be noted that their data include unoccupied burrows, whereas our data concern only live specimens. Nilsson-Cantell (1978) also reports more than 100 T. lampas females in large sized shells (Buccinum undatum and Neptunea antiqua).

It is wholly unclear what causes these variations in prevalence and load among studies, even within the same species of acrothoracicans. As is the case for parasites of hermit crabs, both the general conditions of the habitat and the hermit crab species may possibly have an effect (Høeg and Lützen 1995; Williams et al. 2010). In acrothoracicans, this is further complicated by the same host being able to utilize different species of gastropod shells, and they may well differ in their usefulness for the acrothoracican symbiont. The hermit crab is also likely to change its shell choices as they grow in size. The acrothoracicans will almost certainly survive, when the hermit crab exchanges its shell, because few shells remain unoccupied by hermit crabs for long (Kellogg 1976; Scully 1979; Asakura 1995). In our samples there were next to no empty shells. Reuse of shells could obviously over time lead to an accumulation of empty burrows and possibly of living acrothoracicans, if they live long enough relative to the mean shell exchange period.

**Host sex and size and T. lampas feeding**

**Host Size**

Consistent with our hypothesis (H3), larger crabs had a heavier load of T. lampas. As host size is positively correlated with the shell size, this tendency implies larger shells have more T. lampas females. It is not surprising that a larger substratum also offer the possibility for more T. lampas burrows inasmuch as the symbionts do not seem to interact negatively.

**Host Sex and T. lampas feeding**

Compared to both thoracican and other acrothoracian barnacles, the trypetesids have rather modified cirri that are not extended very far out of the mantle opening (Utinomi 1964; Nilsson-Cantell 1978; Chan et al. 2014a, b), and this raises the issue how T. lampas obtains food. Williams et al. (2011) consistently found remnants of eggs in the digestive tract of T. lampas situated on berried female hermit crabs, and they also recorded a higher prevalence on this sex. In our population, such egg predation cannot be an important food source, because T. lampas was also frequent on male crabs, and the T. lampas carrying crabs comprised even more males (35.5%) than in the total crab population (28.0%); H4 is therefore rejected. Furthermore, specimens on female crabs must obviously be able to utilize other food sources, when the host is not reproductively active, and our results showed that there was no preference for ovigerous over non-ovigerous crabs (i.e., H5 is rejected). The only other food source would be food items lost by the host or food objects in the water circulated through the shell lumen, but its nature and how it is captured remains unknown. The strong bias towards females in the entire hermit crab population could be due to mortality caused by male rivalries (Asakura 1995). But, as explained above, T. lampas will most likely survive the death of its host as long as the shell remains intact and is rapidly reoccupied.

**T. lampas position in the shell**

The larger size of T. lampas specimens in
the columnella part of the shell could be due to more shell material being available for burrow formation, but in general we did not detect a preference for this position. Thus, the columnella may offer better option for growth but there seems to be no competition for space in the shells in our population, which also have a very low load (1.4) compared to some other studies (Williams et al. 2011). Unlike *T. maclaughlinea*, *T. lampas* can flatten its body and may thus be less dependent on a thick layer of shell for burrow formation (Williams and Boyko 2006).

Parasitic rhizocephalan barnacles on hermit crabs always occupy the left side of the abdomen facing the outer shell because they mimic the eggs mass and take advantage of the brood caring behaviour of their host (Høeg and Lützen 1995). We did not record the distance of the *T. lampas* specimens from the shell aperture, but individuals situated far back in the shell are probably assured sufficient oxygen, because hermit crabs use their soft skinned abdomen as an accessory respiratory organ (Vannini et al. 2004). In both male and female crabs, water circulation is assured by beating of the pleopods, which would also benefit the respiration of the symbiont reproduction in *T. lampas*.

We provided evidence for a seasonal reproduction occurring in *T. lampas* (H6), since females was more ovigerous in the summer compared to the winter. But egg or embryo-carrying females were available in good numbers in both summer and winter samples, and we even observed nauplii brooded by females in the November sample. In agreement with this, there was no seasonality in presence of the dwarf males needed for reproduction in this dioecious barnacle species. All this suggests that reproduction takes place throughout the year, at least to some extent. This is somewhat contrary to results of Kühnert (1934) from a North Sea (Heligoland) population, but our result, based only on the ratio of egg/embryo carrying females and measured twice a year, is not an accurate estimate of total reproductive output in the population. The higher summer temperature must almost certainly decrease the time needed for brooding the larvae and also the interval between successive broods, leading to an increased output of propagules from each adult female. The presence of egg/embryo carrying females without males may have two explanations. Firstly, some males may have been exhausted after their last sperm release and dropped away. Secondly, some of the minute and often almost transparent males (Fig. 1) may well have dropped off due to the necessarily rough treatment of cracking the gastropod shell and extracting the females from their burrows.

As expected (H7) the male frequency distribution differed from random, but contrary to the hypothesis, with more females having no males than expected. This seems to indicate, together with a low mean number of males per female (0.63), that sperm competition was not important in this population.

Females with males were more likely to have eggs, either in the ovary or in the mantle cavity. This means that either the presence of males is needed for the development of eggs or females with mature eggs tend to attract males. Furthermore, the number of males was related to the female size, which means that bigger females have more males. This might have various explanations: 1) bigger females provide more space for male settlement, 2) bigger females have a higher chance of carrying eggs to fertilize and 3) bigger females have had a longer time for more males to settle. The two latter explanations seem to be the most reasonable, since males settle in a specific place on the female (Gotelli and Spivey 1992) and therefore the settlement place should not be affected by size.

Female *T. lampas* almost certainly live for several years (Kühnert 1934), while males, being unable to feed, must have a very limited life expectancy. This suggests that females must reacquire males in order to continue to be able to reproduce. Turquier (1972) reported a much higher prevalence of males in *T. nassarioides* (92%) than found in our *T. lampas* population (35.1%). At our study site a large number of females seem to be prevented from reproduction due to lack of males.

In line with our results, Kühnert (1934) also observed *T. lampas* males throughout the year, but especially in May to August. According to Kühnert (1934) the fertilization took place May to September and by males that settled the previous year. This may explain why we did not find any difference in male number between summer and winter.

**CONCLUSIONS**

Knowledge on the taxonomy and phylogeny of the Acrothoracica is finally approaching a firm basis (Kolbasov 2009; Chan et al. 2014b). But our study emphasizes that we still lack a basic
understanding of the life cycle, feeding biology and reproduction of these barnacles. The differences between various studies in prevalence and load are largely unexplained, as is the striking preference for male crabs seen in our data. Feeding biology needs much closer attention in terms of both direct behavioural observations and by ecological approaches. Finally, many details of reproduction remain unclear. We need quantitative data on reproductive output and studies on the mechanism and frequency of copulation in T. lampas or in any other acrothoracican. To answer these questions, we need a broader understanding of acrothoracican relationship to their hosts and of barnacle reproductive system in general (Yusa et al. 2012).

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Additional File 1

Excel file (Trypetesa_Data_2015.xls) containing tabular data on: Total host population (Crab_Total), T. lampas carrying host population (Inf_Pop_Total) and uninfected hosts population (Uninf_Pop_Data), the different parameters are used in the statistic tests. Furthermore the excel file contains a sum-up sheet with host-barnacle relation data (Sum_up_pop_data) as well as a sheet for load frequency distribution of T. lampas females on host (Tl_Load_Distrb_Histogram) and a sheet for T. lampas male load on females (Male_frequency_distrb_Histogram).