The role of pleopods during locomotion in epibenthic crustaceans

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Cover: *Cherax albertisii*, picture made in the lab by Florian Goldenberg.

Contents

Summary

Little is known about the use of pleopods during walking. In this pilot study the use of pleopods during locomotion will be studied in 5 species of epibenthic crustaceans. The behaviour of the animals was recorded during curve-walking, slope-walking and in a non-manipulative control set-up. The curve walking experiment only showed changes in locomotion in the shrimp *Crangon crangon*. Slope walking experiments showed an increase in pleopod use in *Crangon crangon, Cherax albertisii and Homarus gammarus*. These results indicate that pleopods are mainly mobilised when extra thrust is needed and that this is different in all species.

Finally relations between animal size, weight, pleopod size and pleopod use were established.

Introduction

Walking in crustaceans has so far mainly been studied in crayfish using a variety of methods. In several studies the crayfishes had to walk on a treadmill, tethered or untethered. In other studies free-walking animals were used. In all studies the animals were viewed from above (Fig. 1A), the same way as insects are studied in similar projects.

One of the important results of these studies is that each pair of legs has a distinct role. Legs 2, 3 and 4 pull the body forward and legs 5 push the body. Legs 3 and 4 generate stabilisation and lift, legs 4 also produce the largest proportion of the propulsive force (Jamon & Clarac, 1994)(Fig. 1B).

Curve walking experiments on crayfish walking along a curved path showed that the outer legs 3 and 4 move in a different direction than the inner legs 3 and 4 (Domenici et al, 1998). This was not seen for legs 2 and 5. There were no differences found in stride length or frequency. A curve walking experiment on tethered crayfish walking on a treadmill and stimulated to turn showed something similar (Cruse and Silva Saavedra, 1996). In this study the step directions of legs 2, 4 and 5 were different and that the posterior and anterior extreme position of inner leg 5 almost coincided. These differences are probably due to the different methodologies and/or the different species used. If curve walking is studied in tethered animals it cannot be seen how the movements of the legs affect the position of the body. Previous research did show that the pleopods of crustaceans could be used for locomotion in pelagic crustacean, reproduction, carrying eggs and in ventilation in burrowing shrimps like *Callianassa subterranea* (Stamhuis et al,1996).

The ipsilateral pleopods of many malacostracans, including *Palaemon* sp., *Procambarus clarkii* (Lochhead, 1961), and *Homarus* sp. larvae (Laverick et al.,1977), beat in a metachronal pattern.

Studies on the pleopod rhythm of *Procambarus* sp. (Hughes & Wiersma, 1960) showed that pleopods on either side of a segment can beat in a different frequency. This behaviour is known for only a few species. In fast swimming species like *Pandalus leptocerus* this behaviour is probably even impossible because contralateral pleopods are attached to each other by small hooks on a extremity on the inside of the endopodiet (Bell,1905). In this way, the pleopods can beat more effectively.
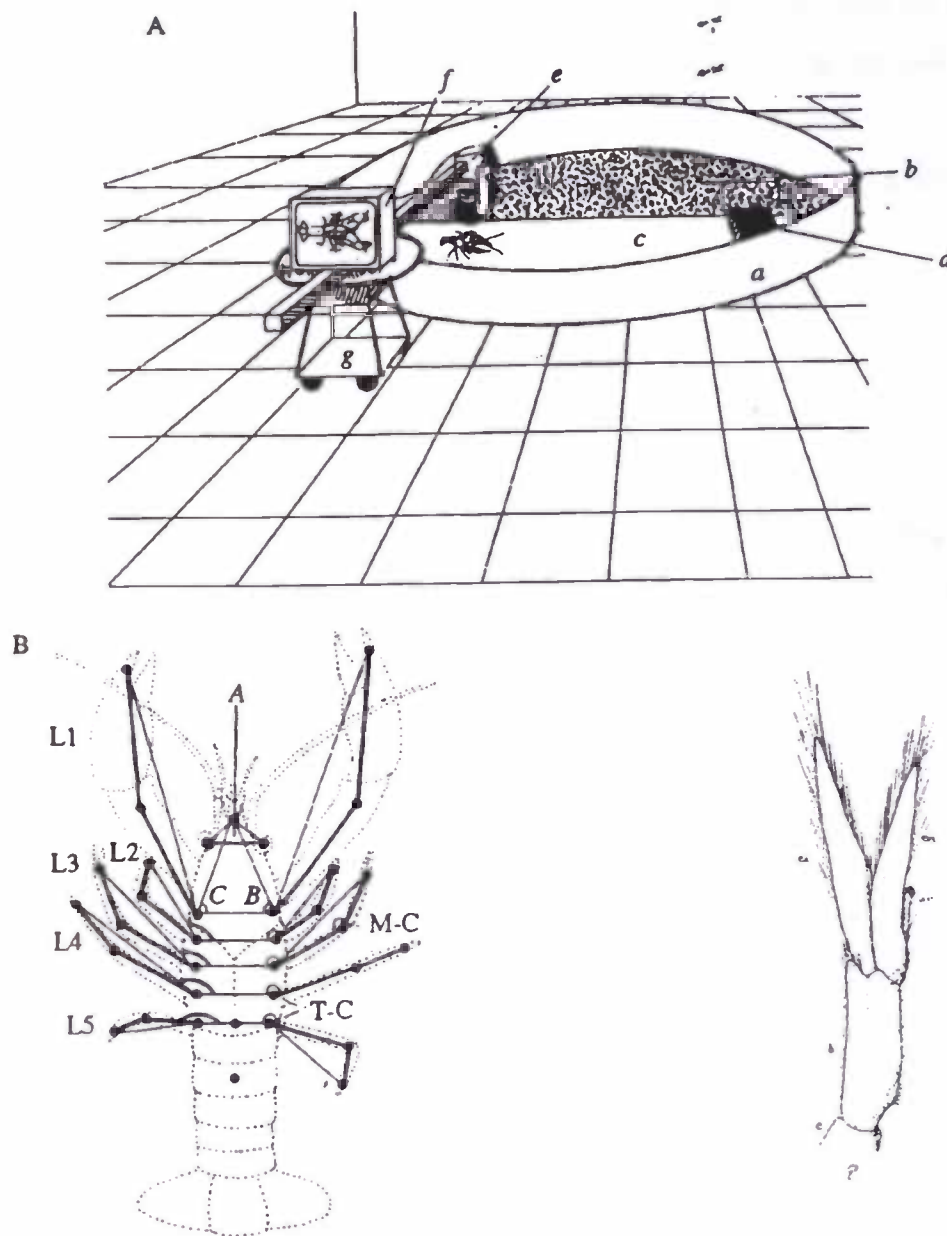
Fig.1: A) An experimental set-up in which a freely walking crayfish is observed from above. B) A top-view of a crayfish (Jamon & Clarac, 1994). C) A rostral view of the pleopod of a pelagic crustacean (Bell, 1905)

After the study of Dominici, Jamon and Clarac (1998) the question was raised whether pleopods would play a role in walking in crayfish and especially in manoeuvring.

The ability of contralateral pleopods *Procambarus clarkii* to beat in a different frequency could enable the crayfish to use the pleopods for steering in manoeuvring tasks.

Homarus sp. has been known to use pleopods for climbing and 'gliding'. Gliding means that the pleopods produce a current that is too weak for swimming but strong enough to decrease

the contact between the walking legs and the substratum. In these cases the pleopods could be used to create an upward force that lightens the task of the walking legs and, in case of gliding maybe increase the speed of the animal.

In order to study this, 5 species of malacostracans were observed during different locomotion tasks.

Research question

What is the role of pleopods during walking of shrimps, prawns, crayfishes and lobsters in different circumstances (curve walking and slope walking)? Can this be related to the natural way of life of the animal, when known?

Materials and methods

Experimental animals

The species used in this study were *Crangon crangon* (brown shrimp), *Palaemon elegans* (common prawn), *Palaemonetes elegans*, *Cherax albertisii*, *Procambarus clarkii* (red swamp crayfish) and *Homarus gammarus* (common lobster).

These species were chosen because of their availability through regular aquarium shops and fisheries, and because of their size differences.

Twenty brown shrimps (*Crangon crangon*) were caught in the Wadden Sea with a pushnet. The shrimps were kept in an aquarium of 60x14x30 at 18°C and the bottom of the aquarium was covered with sand. The water had a salinity of 30-32 ‰ and was constantly filtered and aerated. The length of the animals varied between 3 and 4 cm (Table 1).

Four prawns (*Palaemon elegans*.) were caught in the Wadden Sea several months before the observations started. During the research period another prawn was caught in the Grevelingen, this one is of a different species, probably *Palaemonetes varians*.

Prawns have paddle-shaped pleopods, like the one presented in fig. 1C. The prawns used in this study were between 3.5 and 4.2 cm in length (Table 1). The prawns were kept in a similar aquarium and at the same temperature as the shrimps. The aquarium had a sandy bottom and contained some rocks.

The two species of crayfish were bought in a local aquarium shop. Four specimens of *Cherax albertisii* as well as the three specimens of *Procambarus clarkii* were kept in separate aquariums that had a sand-covered bottom and a shelter. Two crayfish (one *P. clarkii* and one *C. albertisii*) shared a sixty-litre aquarium that was divided in two compartments of similar size. The aquariums were filled with fresh water and were constantly filtered and aerated.

Cherax albertisii originates from rivers in New Guinea (Holthuis, L.B., 1939). The animals used in this study were between 7 and 9 cm in length.

The crayfish *Procambarus clarkii*, the Red Swamp crayfish, originally comes from North America.

The ones used in this study were all about 8 to 10 cm. in length.

The common lobsters (*Homarus gammarus*) were purchased from a local sea food distributor.

Homarus gammarus is very common in coastal waters of Europe. It can get up to 1 meter in length but due to heavy fishing only very few animals get to such a size (Hayward, 1996). The animals that were used in these experiments were between 20 and 25 cm. in length.

The lobsters were kept separately in large seawater aquariums with a sand-covered bottom and a shelter. The water had a salinity of 30-32 ‰ and was constantly filtered and aerated.

Table 1: Weights and measurements

Crustacean	Total length (cm)	pleopod length (cm)	weight in air (g)	Weight in water (g)
Crangon 1	3.8	0.6	0.36	0.01
Crangon 2a	3.5	?	0.29	0.03
Crangon 2b	3.5	0.5	0.31	0.02
Palaemon 1	4.2	0.9	0.85	0.04
Palaemon 2	3.5	0.7	0.45	0.02
Palaemon 3	3.6	0.7	0.56	0.025
Cherax 1	8.8	1.1	14.07	1.02
Cherax 2	8.5	1.5	12.08	0.92
Cherax 3	8	0.8	9.86	0.84
Cherax 4	7.4	0.8	8.65	0.73
Procambarus 2	8	1.7	15.22	1.25
Procambarus 3	10.1	2.3	34.85	4
Procambarus 4	10	2	29.58	3.15
Homarus 1	25.6	3.9	554.4	49.15
Homarus 2	26	3.5	538.69	41.8
Homarus 3	23.9	3.5	461	47

All animals were fed twice a week. The marine species were fed with small pieces of fillet of haddock and salmon. The freshwater species were fed on frozen *Artemia* sp.

An ethogram was made for every species. This information was obtained by observing the individuals and recording the behaviour and categorising it in different states and events. States take a certain amount of time and can therefore be used to record the duration of behaviour. Events are instantaneous and short lasting. (Altmann, 1973) In this study both states and events were used because this enabled recording of two or more types of behaviour

that occurred at the same time. Types of behaviour that occurred during another type of behaviour (e.g. mouthpart movements during walking) were recorded as events. To be able to compare the results of the different species the same states and events were used in all ethograms as often as possible. The ethogram was then used to observe the behaviour of the different animals in three different experimental conditions: control experiments, curve-walking experiments and slope-walking experiments.

Of the crayfishes and lobsters every individual was numbered and measured (Table 1), marking them was not necessary because they were housed in different aquariums.

The shrimps and prawns were divided in different size-classes, since they could not be identified individually. Several marking attempts were made with a number of waterproof marking liquids, but the results were not reliable. In order to keep the animals intact and healthy no harsher methods were used. Still 5 shrimps died in the first weeks after the marking attempts, but there is no evidence for a causal relation.

The shrimps were divided in the following size-classes: $1 = >3.75$ cm; $2 = (3.25 - 3.75)$ cm; $3 = < 3.25$ cm in total length. Each size-class consisted of at least three individuals. The three specimens of *Palaemon elegans* were divided in two size classes: class $1 = (3.75 - 4.25)$ cm consisted of one individual and the class $2 = (3.25 - 3.75)$ cm consisted of two individuals.

The *Palaemonetes varians* was of the same length as the class 2 prawns, but it was not put in the same class because it belonged to a different species, and it was not known if there would be differences in behaviour between these species.

Pr. clarkii and *H. gammarus* were observed in at night, usually between 8 and 10 PM, because they are nocturnal. During night observations the aquarium was illuminated with a red light, assuming that the crustaceans used in this study were among the many sea-living animals that don't have the ability to see red light.

The other three species were observed during daytime between 10 AM and 5 PM.

Control experiment

Every individual of crayfish and lobster and every size-class of shrimp and prawn were observed three times for a period of 30 to 60 minutes. During these observations the shrimps, prawns and crayfish were in the aquariums in which they were housed. The lobsters were observed in a special observation aquarium. The states and events were recorded with an eventcollector. On the eventcollector every button represented a state or event. The

eventcollector had an accuracy of 0.1 second. The data were transferred to a computer program and stored. The data were then processed to timeshares per state in percentages of total time (Microsoft Excel) and presented in bar charts together with the data from the other two experiments (SigmaPlot). This way the control observations can easily be compared to the curve walking and slope walking observations. A T-test for 2 independent samples and One-Way ANOVA (plus Scheffés Post-Hoc test for more than 2 samples) were used for testing for significant differences within and between species and set-ups. Prior to these analyses the results were transformed with a root-arcsine-transformation to make the data normally distributed.

The results from the control observations were used to find out if behavioural states were over- of underestimated if the animal had been out of sight during a certain period of time. A Pearson's bivariate correlation test was used to see if there was a significant correlation between the percentage of time that the animal was out of sight and the percentage of time the animal spent of the different behavioural states. For every species the data per state was tested against the percentage of time spent out of sight.

Curve-walking experiment

Halves and quarters of cylinders of 15 and 20 cm in diameter and 25 cm in height were placed in every crayfish aquarium and the observation aquariums of the lobsters, shrimps and prawns to make a curved path (Fig.2). There was one observation aquarium for the shrimps and prawns. Of every size group of the shrimps and prawns only one individual was used and only one *Pr. clarkii* was observed in this set-up.

The individuals were observed while walking along this path. Special attention was paid to the use of pleopods and the position of the abdomen in comparison to the position of the cephalothorax. The observations took 30 minutes and every form of behaviour was recorded. The eventrecorder was not used in these observations. In stead the observations were registered by hand, to be able to make notes on different states or events that were not observed during the control observations. A few states and events were added to the ethograms. The data were processed in the same way as the data from the control observations.
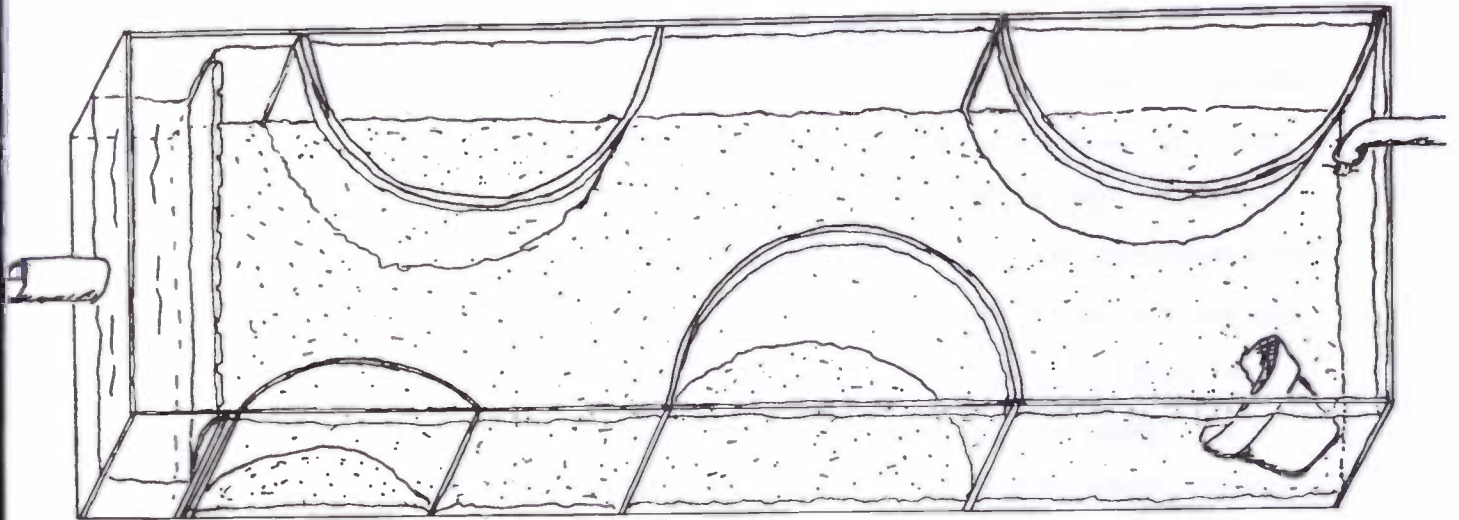
Fig. 2: Top-view of the curve-walking set-up

Slope-walking experiments

For these experiments the animals were observed in an observation aquarium. There was one observation aquarium for the crayfishes, one for the shrimps and prawns and one for the lobsters. Each aquarium contained a slope of which the angle could be adjusted. To observe if the behaviour on the slope changed with size within a species the smallest (Cherax 4) and the largest (Cherax 1) individual of *Ch. albertisii* was observed. Of the other species only one individual was observed; these were Crangon 2, Paleamon 3, Procambarus 3 and Homarus 1. The slopes in the crayfish and the shrimp/prawn aquarium were divided in segments that were held together by hinges. By flipping back the lowest segment of the slope the angle of the slope would increase with 5 °. The angle of the slope could be varied between the 10 and the 45 ° (Fig. 3).
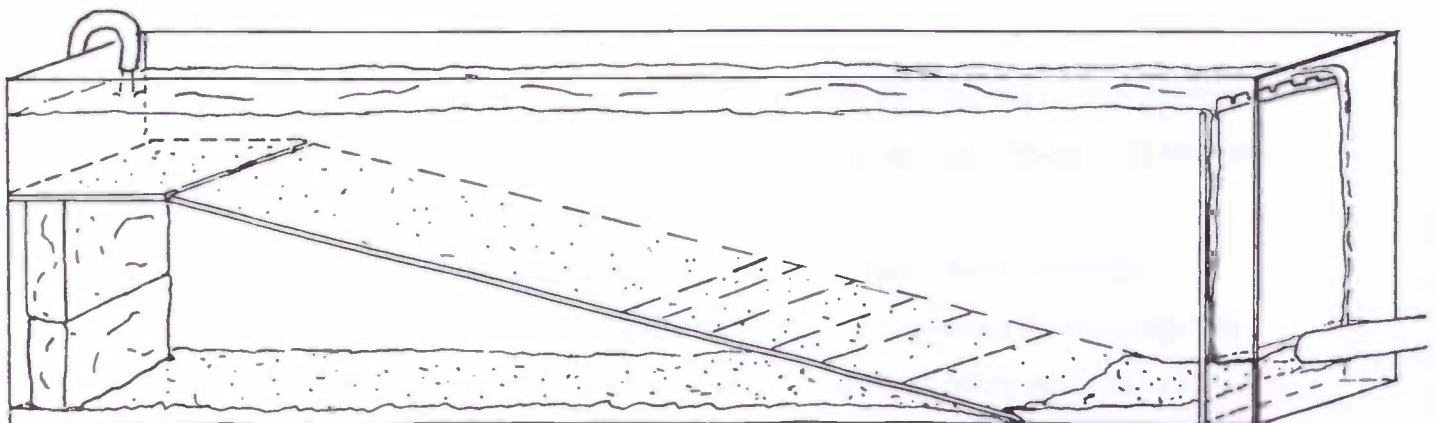

Fig. 3: Lateral view of the slope-walking experiment.

The slope in the lobster aquarium was made out of one piece and was a lot shorter than the other two slopes. This was done to prevent it from sagging. This also meant that the slope was

not more than two times the length of the lobster. All the slopes were provided with an anti-slip layer by painting them with quick-drying paint and covering them with dry fine sand before the paint had dried. During all experiments the lowest part of the slope was covered with sand from the aquarium floor to create a more gradual transition. The animal had to walk up the slope at least three times at all the different angles. The resulting behaviour was also recorded by hand and processed to timeshares per state in percentages of total time. The percentage pleopod use (both walking with pleopod use and walking with bursts) of total locomotion was calculated for all slope angles to determine a relationship between pleopod use and the slope angle. These values were presented in scatter plots with regression lines.

Weighing and measuring
The animals were weighed in air and water to see if there was evidence for the presence of buoyancy-enhancing structures.

This was done after completing most of the behavioural experiments. By then all of the class 3 shrimps had died as well as one of the class 2 prawns and one *P. clarkii*. Weighing was done on an electronic balance with an accuracy of 0.01 grams. On the scale a stand was placed with a magnetic foot, a plate of iron was placed between the scale and the foot (Fig. 4). The animals were tied to a nylon fish line, the other end of the fish line was attached to the stand in a gallows-like configuration. In this way, the animal could be weighed in a bucket of water that was standing next to the scale as well as in air. To prevent them from moving while they were weighed or measured the animals were anaesthetised with a chloroform-(sea)water mixture (1:500).

The shrimps and prawns were anaesthetised to a level in which they did not react to external stimuli after about 20 min. The crayfish took about half an hour and the lobsters took between 45 and 60 minutes till they were dizzy enough to be handled.
The animals were taken out of the anaesthetising vessel with a metal hook. After the measurements the animals were immediately put back into their aquarium for recovery. The shrimps and prawns were first put in a separate to protect them from being attacked by the other shrimps and prawns, while they were still dizzy.

Fig. 4: schematic picture of the set-up that was used for weighing the crustaceans
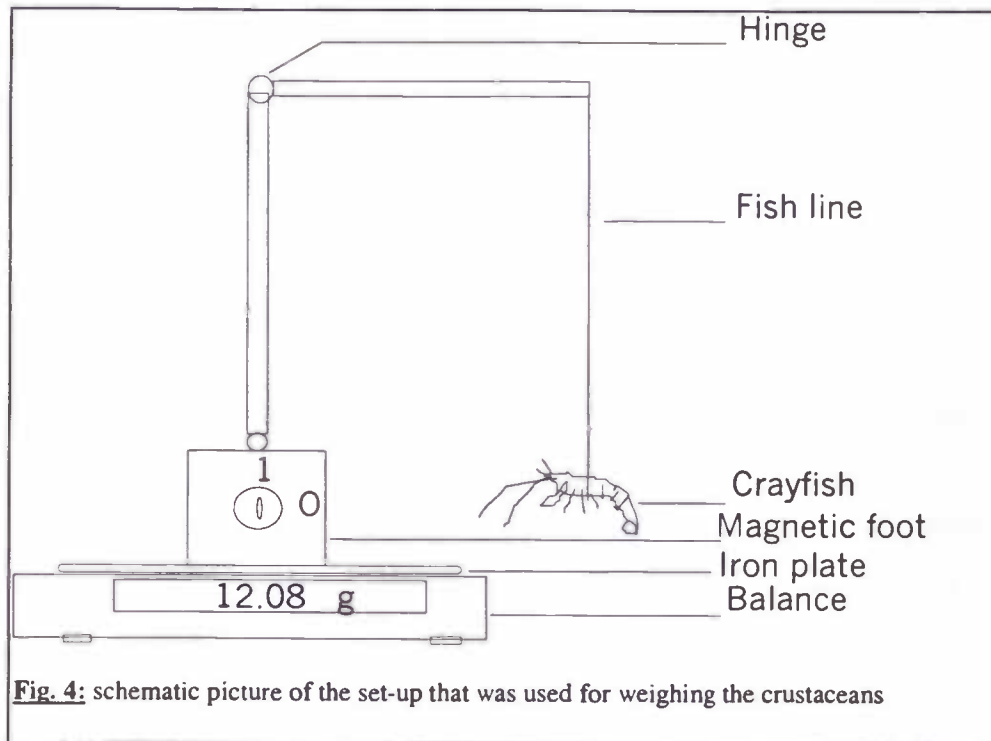
To see if much adhering water (from for instance the gills) was still in the animals when they were weight a test was done with two dead crayfishes. These crayfishes were weighed one just after taking them out of the water. After that they were put on a table to dry and weighed every half-hour. Since weight-loss stay the same over the whole course (fig. 13, appendices) and there was not much water on the table, the weight-loss was mainly caused by evaporation. The adhering water had probably left the body before the first measurement.

Results

Ethograms

The ethograms can be found in table 2 on page 15.

Control experiment

According to the Pearson's bivariate correlation test there are no significant differences between the time spent out of sight and the time spent on the different behavioural states. The correlation coefficients and the P-values can be found in table 7 in the appendices.

In *C. crangon* there seems to be a positive correlation and in *Ch. albertisii* a negative correlation between size and the percentage of time spent on locomotion (Table 3a and 3c). Both these trends are not significant. There is only one significant difference within a species, Procambarus 1 spent significantly more time on vertical walking than Procambarus 2 (2.1% *vs.* 0.1%, Table 3d). There were no significant differences found between *Paleamon elegans* and *Palaemonetes varians*, therefore the prawns will now be treated as one species and referred to as *Palaemon*.

C. crangon is the only species in which Vertical walking is not observed and in this species all Vertical walking with pleopods was performed against the aquarium walls. In the other species pleopod use during vertical walking varied between 32% in *Palaemon* and *Pr. clarkii*, 51% in *Ch. albertisii* and 85% in *H. gammarus*.

Walking with pleopod use as a fraction of total locomotion is significantly higher for *C. crangon* in comparison to the other species (p<0.05) (fig. 5)

Table 2: Ethogram
Table 1a shows all the behavioural states. Every state has a code that is used in other tables and graphs. Columns 4 to 8 show in which species that state was found. The names of the species are abbreviated; Crangon, Palaemon, Cherax, Procambarus, Homarus.
The last column shows in which experiment a certain state was observed. Table 1b shows the events that were found.

2a

States	Code	Description	Cra	Pal	Che	Pro	Hom	Experiments
Walking	W	2nd to 5th pair of pereiopods, contact with substrate	X	X	X	X	X	All
Walking with pleopod use	P	2nd to 5th pair of pereiopods and the pleopods (constantly)	X	X	X	X	X	All
Vertical walking	Wv	Walking on substrate steeper than 45 °	X	X	X	X	X	All
Vertical walking with pleopod	Pv	Using pleopods while walking on substrate steeper than 45°	X	X	X	X	X	All
Walking with bursts	W b	Using pleopods on and off while walking	X	X	X	X	X	Curve & slope
Pleopods right	Pr	Using only right pleopods while walking	X					All
Pleopods left	Pl	Using only left pleopods while walking	X					All
Swimming	Z	Moving with pleopods without contact with substrate	X	X				All
Resting	R	No visible motion except antennae	X	X	X	X	X	All
Grooming	G	Cleaning body with 2nd to 5th pair of legs and 3rd maxillipeds	X	X	X	X	X	All
Probing	S	In and on the substrate with 2nd and 3rd pair of pereiopods	X	X	X	X	X	All
Burying	U	Using pleopods to bury itself in the sand, antennae stay visible	X					All
Bulldozing	B	Walking while pushing sand using 3rd maxillipeds and 1st to 3rd pereiopods			X		X	All
Pushing	Push	Pushing against a solid object			X	X	X	Curve & slope
Falling	Fall	Backwards, landing on abdomen			X	X	X	Curve & slope
Turning	Turn	Making a sharp turn				X	X	Curve & slope
Fanning	F	Moving sand by rapidly moving the pleopods				X	X	Curve & slope

2b

Events	Code	Description	Cra	Pal	Che	Pro	hom	Experiments
Mouthparts	*m*	Moving (several) of the mouthparts	X	X	X	X	X	All
Beating	*b*	Making a few pleopod beats	X	X	X	X	X	All
Interaction	*i*	Contact with other individuals	X	X	X*	X*		All
Turning	*t*	Making a turn of at least 90 °	X	X	X	X		Curve & slope
Lifting	*l*	Lifting itself agianst a vertical object sideways or headfirst			X	X	X	All
Lowering	*d*	Getting back on the floor after lifting itself.			X	X	X	All
Swinging	*s*	Rostro-caudal movements of 2nd, 3rd, 4th or 5th pair of pereiopods				X	X	All

* Only for animals that shared an aquarium (see p.5)

Table 3: The percentage of time spent on the different behavioural states in the control observations. Every table contains the data of all individuals/size-class of a species.
W=walk, Wv=vertical walk, P=walk with pleopod use, Pv=vertical walk with pleopod use, Z=swim, R=rest, G=groom, U=bury, S=scan, B=bulldozer, F=fan.

3a) *Crangon crangon*

	W	Wv	P	Pv	Z	R	G	U	S	B	F
Cra1	11.9	-	3.43	2.661	2.126	62.25	4.646	0.865	12.12	-	-
Cra2	2.344	-	0.984	2.298	1.33	78.83	11.46	0.072	2.69	-	-
Cra3	0.26	-	0.037	0	0	86.76	11.35	0.101	1.489	-	-
Average	4.836	-	1.484	1.653	1.152	75.95	9.151	0.346	5.434	-	-

3b) *Palaemon*

	W	Wv	P	Pv	Z	R	G	U	S	B	F
Pal1	21.44	0.728	4.85	1.892	18.62	2.594	24.3	-	25.57	-	-
Pal2	15.86	0.728	1.975	0.295	37.67	2.562	16.86	-	24.06	-	-
Pal3	15.27	4.367	3.52	0.599	40.48	18.21	7.974	-	9.575	-	-
Average	17.52	1.941	3.448	0.929	32.26	7.788	16.38	-	19.74	-	-

3c) *Cherax albertisii*

	W	Wv	P	Pv	Z	R	G	U	S	B	F
Che1	10.18	0.142	0	0.024	-	41.07	2.257	-	42.94	0.234	-
Che2	15.37	0.275	0.281	2.693	-	33.92	17.74	-	29.31	0.405	-
Che4	25.16	1.695	0.352	2.572	-	21.95	6.904	-	37.13	2.442	-
Average	16.9	0.704	0.211	1.763	-	32.31	8.967	-	36.46	1.027	-

3d) *Procambarus clarkii*

	W	Wv	P	Pv	Z	R	G	U	S	B	F
Pro1	39.56	2.145	0.68	0.968	-	22.12	0.416	-	34.11	-	-
Pro2	39.53	0.11	0.517	0.089	-	17.16	4.469	-	38.12	-	-
Average	39.54	1.127	0.598	0.529	-	19.64	2.443	-	36.12	-	-

3e) *Homarus gammarus*

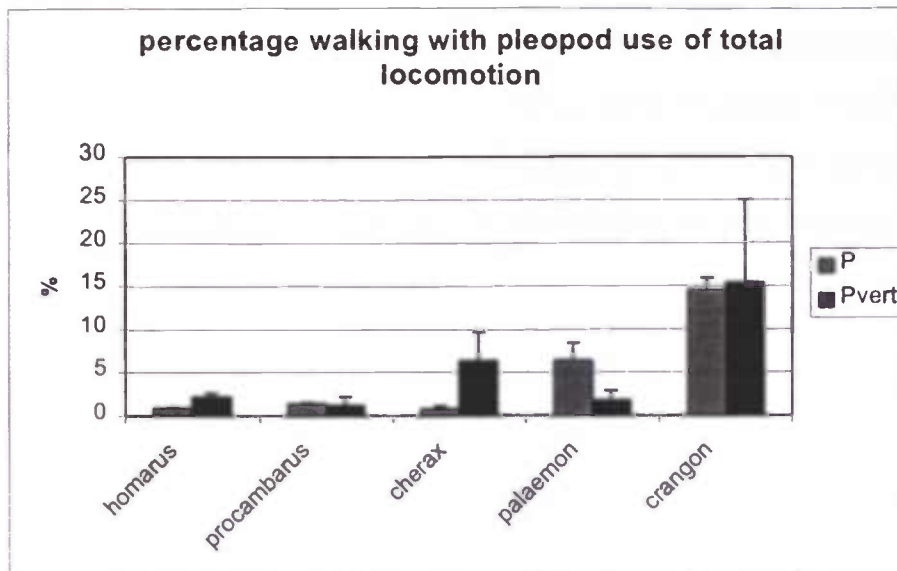|	W	Wv	P	Pv	Z	R	G	U	S	B	F
Hom1	27.29	0	0.309	0.661	-	31.27	18.23	-	20.01	2.016	0.205
Hom2	11.83	0.158	0.116	0.187	-	36.35	11.09	-	25.87	12.85	0.187
Hom3	29.61	0	0.233	0.865	-	8.356	2.251	-	22.86	30.27	2.081
Average	22.91	0.053	0.219	0.571	-	25.33	10.52	-	22.91	15.05	0.824

Fig. 5: Horizontal and vertical walking with pleopod use (P and Pv) as a percentage of total locomotion based on the data of the control observations. The asterisk (*) indicates a significant difference from all other species.

Other states in which *C. crangon* is significantly different than the other species are Walking and Sitting. Walking as a percentage of total time is lowest (4.8%) and Sitting is highest (76%) in *C. crangon*. Scanning is in *C. crangon* significantly lower than in the crayfishes and lobsters (Table 3).

The crustaceans with the ability to swim use pleopods more often during locomotion that the non-swimming crustaceans do (Fig 5). Palaemon spent significantly more time on swimming than *C. crangon*.

Bulldozing has only been observed in *H. gammarus* and *Ch. Albertisii* and the percentage Bulldozing as a fraction of total time was significantly higher in *H.gammarus* (15% vs. 1%). Also pleopod use during Bulldozing was highest for *H. gammarus*.

Pr. clarkii spent significantly more time on walking than *Ch. albertisii*.

Curve walking

C. crangon, *Ch. albertisii* and *Paleamon* spent less time on resting and more time on walking when compared to the control observations (fig 6).

In *H. gammarus* resting is higher and walking is lower than in the control observations.

An important observations in the curve walking experiments is that *C. crangon* uses right pleopods while making a left turn and vice versa in 6% of total horizontal locomotion. This

behaviour was not observed in the control observations or in any of the other species. On three occasions *Ch. albertisii* seemed to slightly turn the abdomen towards the curve, but this was difficult to see. It didn't use pleopods during these occasions. Both *Ch. albertisii* and *Pr. clarkii* spent more time on Vertical Walking with and without pleopod use during the curve walking observations than during the control observations, because the crayfish climbed up between cylinders and the aquarium walls.
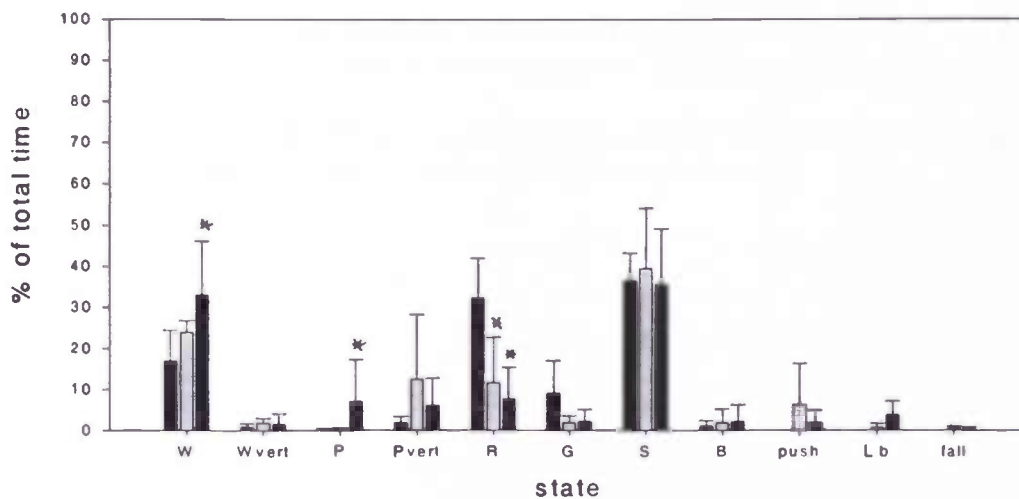
A: Crangon crangon
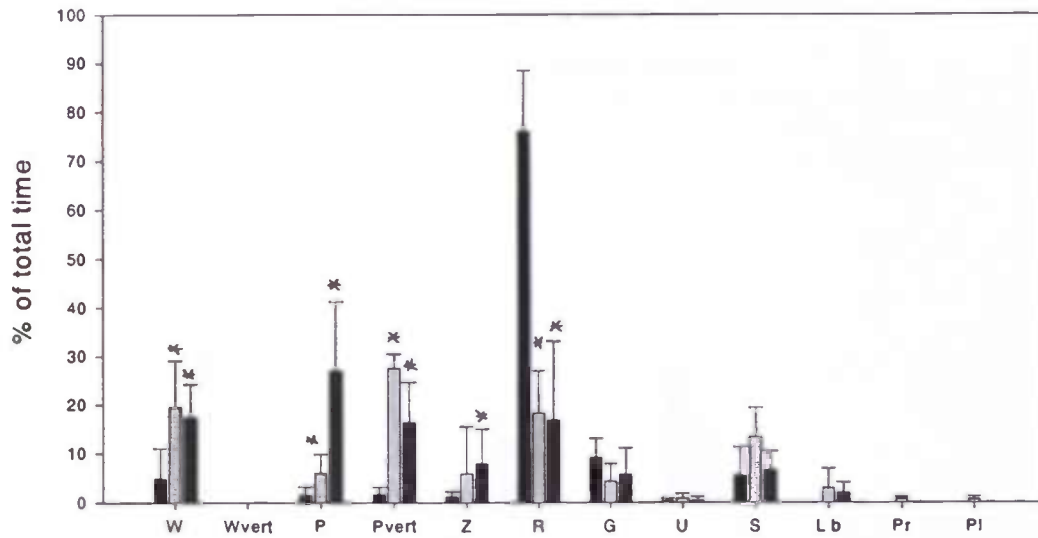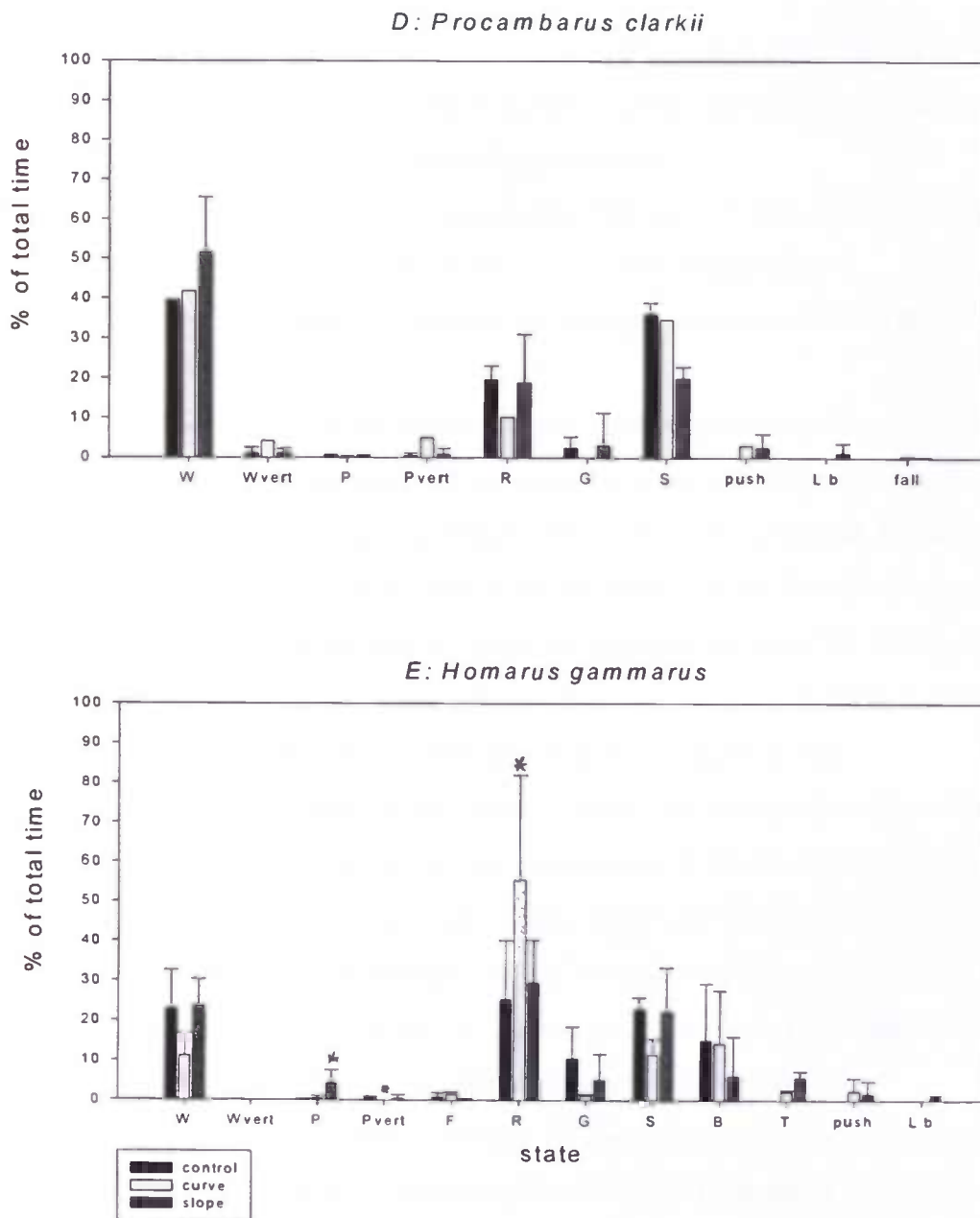
B: Palaemon
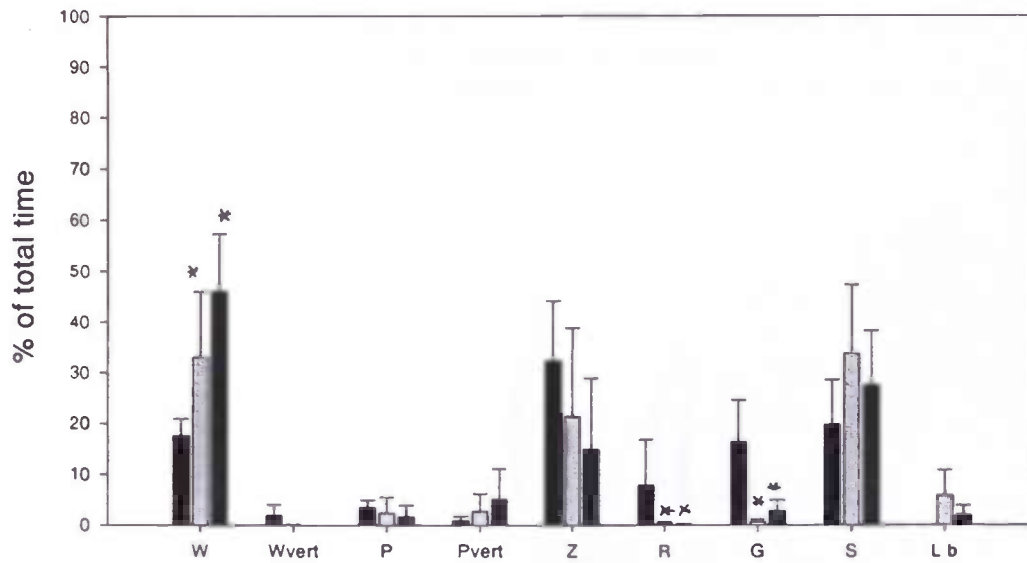
C: Cherax albertisii

state

Fig. 6: Percentage of time spent on all the states in the three experimental set-ups. The asterisks indicate a significant difference with the control situation. These data can also be found in table 6 in the appendix.

W=walk, Wvert=vertical walk, P=walk with pleopod use, Pvert=vertical walk with pleopod use, Z=swim, R=rest, G=groom, U=bury, S=scan, B=bulldozer, F=fan.

Slope walking

Resting was again lower and walking higher than in the control observations for *C. crangon*, *Palaemon* and *Ch. albertisii* (fig. 6). In *H. gammarus* the time spent on resting and walking was about the same as it was in the control experiments.

For *C. crangon*, *Ch. albertisii* and *H. gammarus* Walking with pleopod use was significantly higher in the slope observations than in the control observations (fig. 6).

This was caused by an increase of pleopod use during locomotion both on the slope as well as off the slope.

The two individuals of *Pr. clarkii* that were used for the control experiments could not be used for the slope walking experiments (one had died, the other was limping), therefore two other animals of the same species were purchased. One of these, Procambarus 3, was then used in the slope experiments. There are no data of the behaviour on the 40 and 45° slope, because Procambarus 3 did not recover properly from the narcosis (see page 12). The behaviour of the lobster *H. gammarus* on the 45° slope was not observed, because the lobster seemed to have lost interest in the slope and the research period was coming to an end.

The results for Cherax1 showed an increase in pleopod use during all angles of the slope when compared with the results from the control experiments. A linear regression showed a strong trend towards more pleopod use with increasing slope angle (Fig. 7c). On the 10° slope pleopods were used during (on average) 25% of the total locomotion time on the slope, this was only walking with bursts. On the 45° slope pleopod use was 100%, about 45% of this was walking with bursts and 55% was walking with constant pleopods.

The results from the other animals including Cherax 4 did not show a similar trend. In Cherax 4 pleopod use was high during all angles, even on the level ground in front of the slope. On the 25°, 30° and 35° slope it constantly used its pleopods (Fig. 7d). On the 45° slope pleopod use made up only 40% of total locomotion and this consisted for about 60% of walking with bursts. Off the slope pleopod use during locomotion was then only 2%, while it had been 60% during the 25° slope experiment.

The regression line even showed a slight decrease with increasing slope angle for Walking with pleopod use and Walking with bursts in Cherax 4, Walking with pleopod use in *H. gammarus* and *Pr. clarkii* and Walking with bursts in *C. crangon*. In the other cases the regression line showed a slight increase with increasing slope angle but statistical tests for linear regression showed that the regression coefficient was never significantly different from zero (Fig. 7a-f).
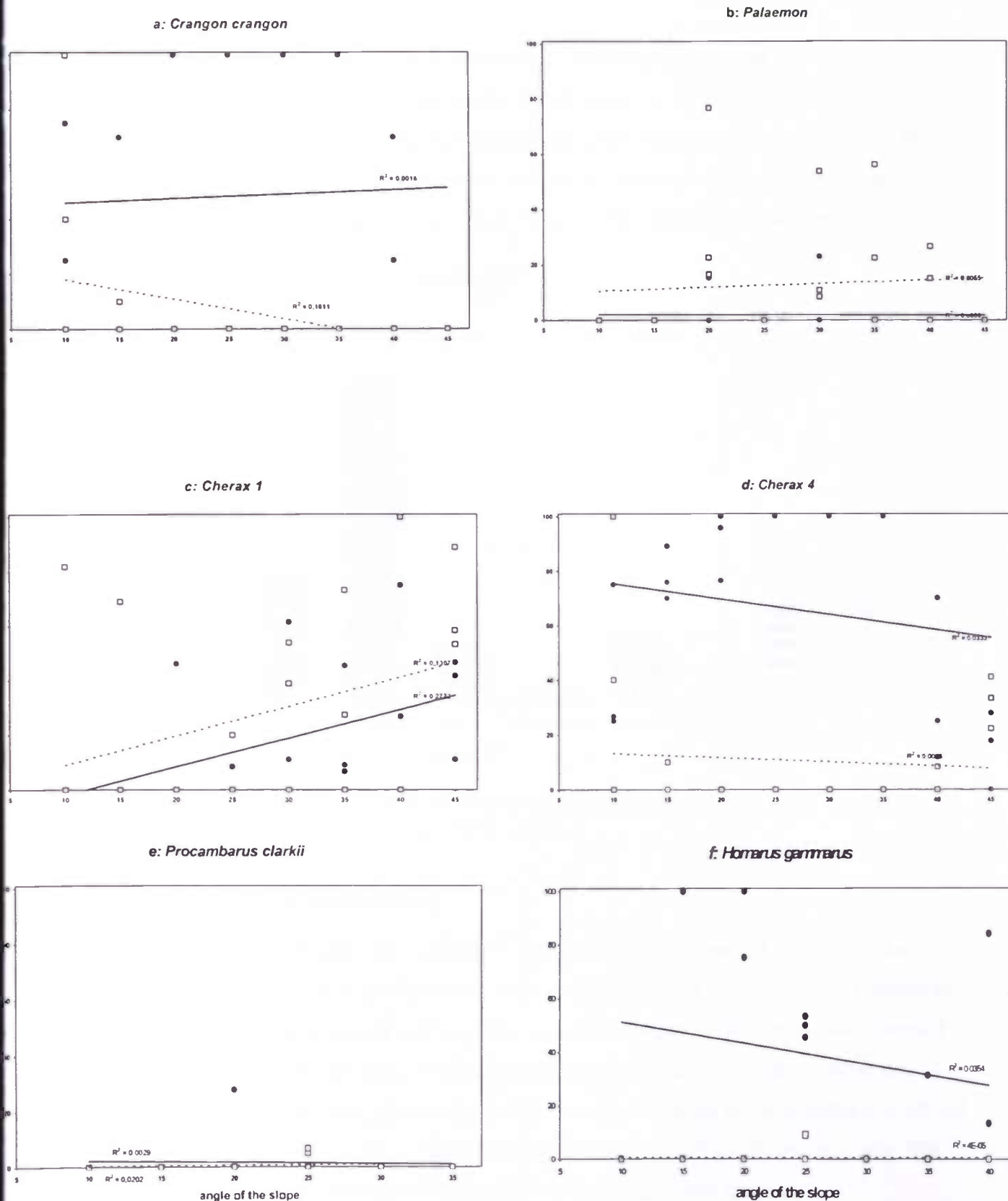
Fig. 7: The black circles and the continuous regression line represent the state walking with pleopods. The white squares and the dashed regression line represent walking with beats. Of both states 3 values go with every angle of the slope. Some of the circles are masked by other circles or by squares.

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In total, pleopods are used during 9 different states. Walking and vertical walking are the only states during which all species use pleopods, though not all to the same extent. Fig. 8 shows for each species during which states it uses pleopods and how much time was spent on that in comparison to total observation time. Slope walking was only seen as a different state in species that used pleopods significantly more often on the slope then off the slope.
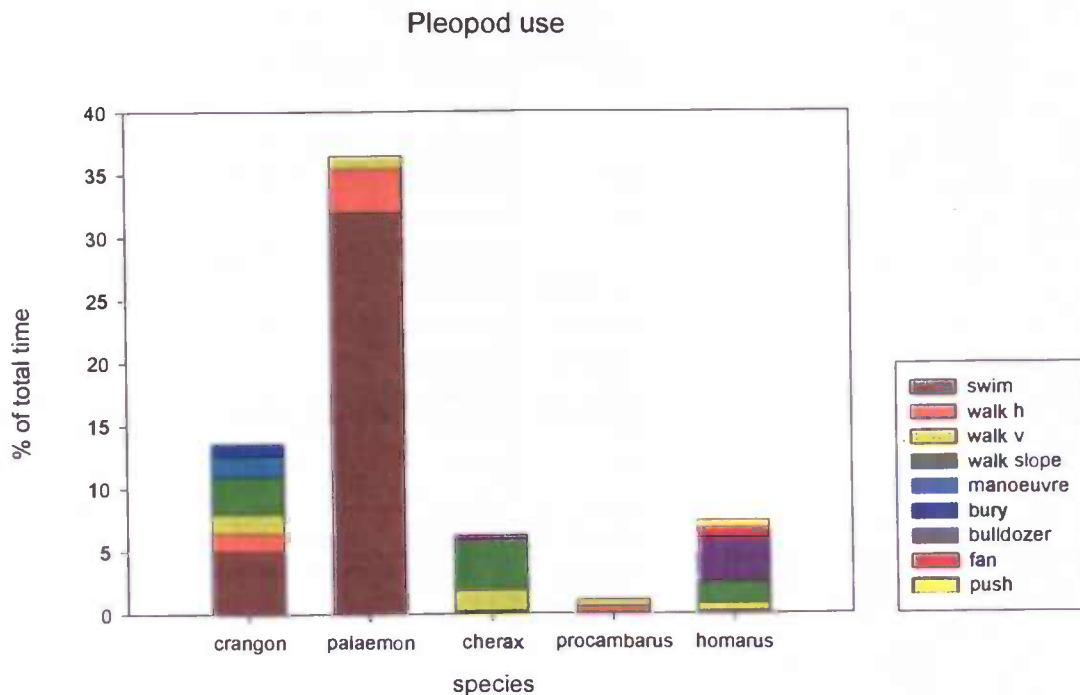
Pleopod use

Fig8: This graph shows the time spent on using pleopods as a percentage of total time. Every bar represents a species and every stack represents a state.

Weight and length relationships

H. gammarus is significantly heavier than *C. crangon*, *Palaemon* and *Ch. albertisii* There are no significant differences in the W(water):W(air) ratio among the species (table 5), therefore the relationship between weight and length in both air and water is the same in all species. In water the large animals (lobsters) are relatively lighter than in air when compared to the smaller animals (Fig. 9). The regression coefficients of both curves are very high (R=0.98 and R=0.99) but the standard error for variable b is 0.31 in the L(total) vs. W(water)-curve, this means that there is no significant difference between the regression curves.

Table 5: This table also contains three calculated ratios.
These ratios are: Pleopod length (L(pleo)): Total length (L(total))
Weight in water (W(water)):weight in air (W(air))
Total length (L(total)):weight in water (W(water))

Crustacean	Ratio L(pleo) : L(total)	ratio W(water) : W(air)	Ratio L(total) : W(water)
Crangon 1	0.157895	0.027778	380
Crangon 2a	?	0.103448	116.6667
Crangon 2b	0.142857	0.064516	175
Palaemon 1	0.214286	0.047059	105
Palaemon 2	0.2	0.044444	175
Palaemon 5	0.194444	0.044643	144
Cherax 1	0.125	0.072495	8.627451
Cherax 2	0.176471	0.076159	9.23913
Cherax 3	0.1	0.085193	9.52381
Cherax 4	0.108108	0.084393	10.13699
Procambarus 2	0.2125	0.082129	6.4
Procambarus 3	0.227723	0.114778	2.525
Procambarus 4	0.2	0.106491	3.174603
Homarus 1	0.152344	0.088654	0.520855
Homarus 2	0.134615	0.077596	0.62201
Homarus 3	0.146444	0.101952	0.508511

To see if the pleopods of the different species were relatively different in length the L(pleo):L(total) ratio was calculated. This is the ratio between the length of the pleopods and the total length of the body (table 5). This ratio shows that *Pr. clarkii* relatively larger pleopods *Ch. albertisii*, *C. crangon* and *H. gammarus* (P<0.05*). Ch. albertisii* also has relatively shorter pleopods than *Palaemon* (P<0.05). In Fig. 10 this ratio is plotted against the data for total pleopod use that were presented in Fig. 8, to see if relative pleopod length was related to pleopod use. There is a trend that species with relatively larger pleopods used them more often. The only species that doesn't follow this trend is *Pr.clarkii* and there is a significant correlation if this species is left out.

Furthermore the two species, in which pleopod use during vertical locomotion is lowest, *Palaemon* and *Pr. clarkii*, are also the species with relatively the largest pleopods.
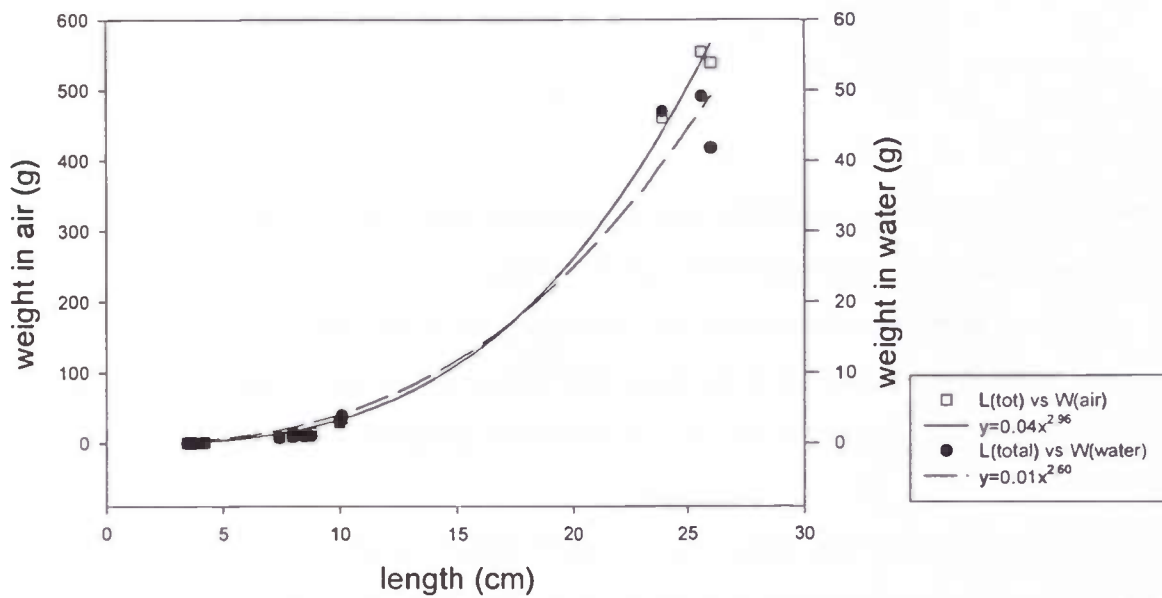
Fig. 9: The weight in air and the weight in water plotted against the total length. Through each plot a curve $y=ax^b$ was fitted
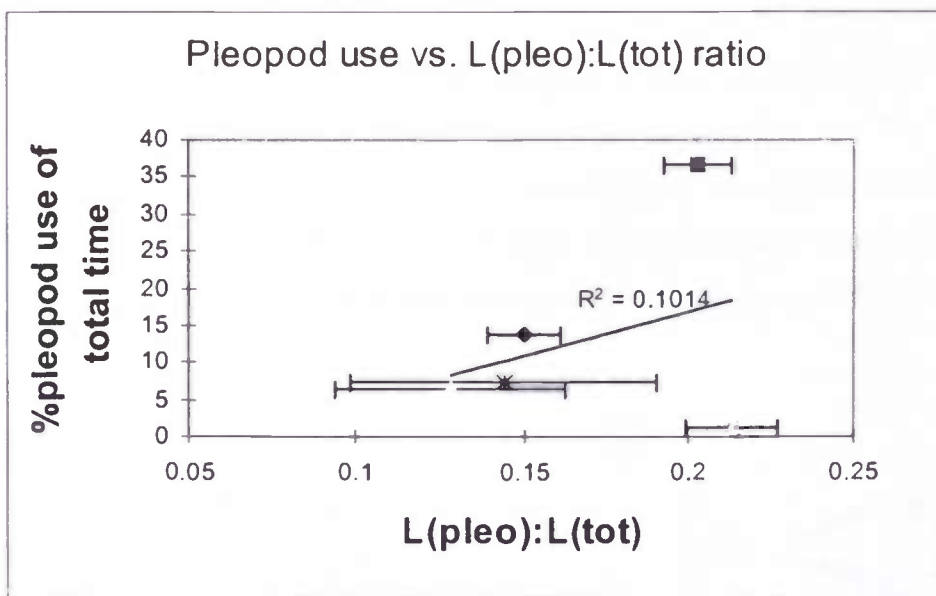

Fig. 10: Total pleopod use (fig. 5) plotted against the relative pleopod length.
◆ = *C. crangon*, ■ = *Palaemon*, Δ = *Ch. albertisii*, × = *Pr. clarkii*, ✳ = *H. gammarus*

Discussion

Experiments

Control observations

Most of the animals were put in a glass aquarium in order for them to get used to the presence of people. Still the crayfish sometimes responded to a sudden move of the observer or of another person. In those cases they lifted themselves against the aquarium wall or were standing still with raised claws. The lobsters were observed in the dark period, but not in complete darkness and they were easily disturbed by a passing shadow. This often resulted in hiding in the shelter.

The thin layer of dark sand, which was put in the Crangon aquarium to enhance the visibility of the legs, had a larger grain size than the fine sand that was used in all the aquariums. The grain size of the dark sand was probably to large for the shrimp to bury themselves in and the brown shrimps could only bury themselves in the few places where the dark sand did not cover the fine sand. The disability of the shrimps to bury themselves in the dark sand made it easier to observe them but it could also have been stressful for the shrimps.

Curve walking

An important difference between the pleopods of *C. crangon* and those of the other 4 species is that they move mainly sideways in stead of downward (fig. 11). This enables the shrimp to use the pleopods for manoeuvring (curve walking). It was often difficult to see if the shrimp was using pleopods on one or on both sides of the body since it could only be observed from the side. Therefore it could be that the states Pleopods right and Pleopods left were underestimated.

Fig.11: Main direction in which the pleopods of A) *H. gammarus* (lateral view) and B) *C. crangon* (dorsal view) beat.

For the prawns, crayfishes and lobsters there were no significant differences found in pleopod use during walking between the curve experiments and the control observations. There were also no actual changes observed in the position of the abdomen or the uropods while walking along a curved path.

Slope-walking

The presence of the slope caused an increase in pleopod use during walking for *C. crangon*, *Ch. albertisii* and *H. gammarus*. This increase was not just on the slope, but could often also be seen off the slope, especially in Cherax 4 and Homarus 1. Cherax 4 spent more time resting than Cherax 1, but when it was walking it was walking very fast and often using its pleopods. Homarus 1 often walked very slowly toward the slope and stood still in front of it. Then suddenly it turned around and walked away with beating pleopods. After 5 to 10 minutes it would do this again. It walked the slope on average less than 2 times an hour. It was very easily disturbed and after an hour or an hour and a half it would completely lose interest. Attempts were made to lure the animals up the slope with food but this was not possibly in a non-disruptive way because the presence of food seemed to trigger them to use their pleopods. Homing behaviour was also tried as a stimulus to get the lobsters and crayfish to walk the slope. This worked for the crayfishes but not really for to lobsters. The lobster did approach the slope more often but especially with the steep slopes it wouldn't go on it. Homing behaviour was successfully used in the studies of Jamon and Clarac (1995) and Domencini, Jamon and Clarac (1998). Two important differences between those studies and this study is that they trained the crayfish to walk towards the shelter and that the crayfish were put in the observation aquarium shortly before it was observed. In this project the animals where often put in the observation aquarium at least an hour before the observation started and were left in that aquarium until all the observations were done. This could take several days. It is still hard to say which method is better since it is not known whether the animals in the other studies where using pleopods.

Comparing species

The control observations showed only one significant difference between the two species of crayfish; *Pr. clarkii* walked significantly more that *Ch. albertisii*. An explanation for this could be that *Pr. clarkii* is nocturnal and *Ch. albertisii* seems to be active both day and night.

Pr. clarkii was always observed in the first hours of the dark period (8-10 PM). It could be that it is extra active in this period.

Ch. albertisii and *H. gammarus* seem to use the same bulldozer technique, still *H. gammarus* uses its pleopods more often during bulldozing than *Ch. albertisii* (fig 9). It could be that *Ch. albertisii* moves a relatively smaller amount of sediment at a time. It could also be that has more grip on the substrate than *H. gammarus*. If this were true than this could also mean that *Ch. albertisii* has more grip on the slope than *H. gammarus* since the same grain-size was used for all slopes. This would also mean that the shrimps and prawn had even more grip of the slope than the crayfishes. On the other hand, this theory cannot explain way *Pr. clarkii* showed no increase in pleopod use on the slope when compared to a level substrate.

C. crangon was the only species that spent more than 50% of the behaviour observations on sitting. This is probably the cause for most of the significant differences between the shrimps and the other 4 species.

C. crangon seemed to prefer vertical walking with pleopods to swimming. Staying close to the substrate probably provides the shrimp with camouflage. The lack of rocks in the aquarium of *C. crangon* could have caused the absence of vertical walking.

Weighing and measuring
The relation between length and weight answers to an $y=ax^b$ equation both in air and in water. In both situations $b < 3$, this means that the lobsters are relatively lighter than the smaller species than they would be if they size would be equally larger than for instance a shrimp in all three dimensions. There is an allometric relation between length and weight. Since the values of b overlap the curves are probably not significantly different and the animals probably don't have buoyancy-enhancing structures.

Though the weighing and measuring went fine, the anaesthetic was not perfect. One prawn and one shrimp didn't come out of the narcosis. In the week after the treatment two *Ch. albertisii* died and another had problems moulting. The new carapace seemed to have hardened to slowly, which left the animal with a bent rostrum and buckled antennae. The animal is still alive and healthy, but it is uncertain whether it will survive a next moult. The *Pr. clarkii* that was being used for the slope experiments behaved differently after the chloroform treatment. It seemed to be unable to straiten its abdomen and it spent a lot of time sitting and hiding. When it walked it seemed out of balance.

Fig. 11 shows that there is a trend that species that use their pleopods often have relatively large pleopods. If *Procambarus clarkii* would be left out of the graph there would be a significant correlation between pleopod use and relative pleopod length. *Pr. clarkii* also used pleopods less often and in fewer situations than *Ch. albertisii*. This could mean that the specimens of *Pr. clarkii* that were used in this study were not representative for this species. It could also mean that *Pr. clarkii* has a different technique for dealing with heavy locomotion tasks like slope walking. It could for instance use a different gait in these situations.

If the crustaceans had structures with a density roughly the same as that of water (e.g. fat reserves, air chambers) than the lobster could have the same weight as the shrimp. This would influence the interpretation of the results of the observations.

Conclusion

All species use pleopods in vertical walking but this is not equally in all species.

In *Ch. albertisii* and *H. gammarus* pleopods used on slopes and during bulldozing. It could be that they use bulldozing for digging out a shelter.

C. crangon is the only one of these species that uses pleopods for manoeuvring and *C.crangon* is also the only one that has pleopods that are mainly directed sideways in stead of downward. The sideways-directed pleopods are specialised for burying itself in the sand.

In short, one can say that pleopods are used to assist the legs mostly when extra power is needed. It depends on the species under what circumstances this is the case.

Further research

* One way to increase the sample size is to use fewer species.

* Flow analyses to (maybe) distinguish between different kinds of pleopod beats.

* <u>Curve walking:</u>

The use of a longer path for the crayfishes and lobsters could perhaps induce manoeuvring with the pleopods or uropods.

Observing the shrimps from above in stead of from the side will provide the observer with a better view of the pleopods and of the movement of the body. This way the effects of manoeuvring with pleopods can be observed more precise.

* <u>Slope walking:</u>

The use of steeper and, in case of the lobster, longer slopes would increase the amount of thrust needed to climb the slope. Maybe then a relationship between the angle of the slope and pleopod use can be established.

Using a slope that is e.g. 50 steps long, will give the slope the same relative length for all species.

Using a slope that fills the aquarium when observing the lobsters, will make sure that all the walking is done on the slope.

* When anaesthetising crustaceans with chloroform make sure to get them out of the chloroform as soon as they stop responding to external stimuli. And put them back in their aquarium as quickly as possible.

Do not anaesthetise animals that have to stay healthy for further research.

Best is to find another anaesthetic that doesn't have lethal side affects. Ether could be a better solution (pers. comm. E.J.stamhuis)

Acknowledgements

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Appendices:

Table 6: The percentage of time spent on each of the states is presented in these tables. Each table represents a species. Of each value the standard deviation is given. An asterisk (*) at the end of a value indicates that there was a significant difference within the species. A (_) indicates that state was not observed in that experiment.

W=walk, Wvert=vertical walk, P=walk with pleopod use, Pvert=vertical walk with pleopod use, Z=swim, R=rest, G=groom, U=bury, S=scan, B=bulldozer, F=fan.

6a *Crangon crangon*

Experiment	State (% of total time)										
	W	P	Pv	Z	R	G	U	S	L b	Pr	Pl
Control	4.84 ± 6.21	1.48 ± 1.75	1.65 ± 1.44	1.15 ± 1.07	75,95±12,51	9.15 ± 3.90	0.35 ± 0.45	5.43 ± 5.82	–	–	–
Curve	19.59 ± 9.50	5.91 ± 3.89	27.55± 2.93	5.85 ± 9.62	18.32 ± 8.72	4.33 ± 3.57	0.86 ± 0.99	13.37± 6.03	2.93 ± 3.97	0.68 ± 0.45	0.61 ± 0.61
Slope	17.39 ± 6.88	26.92± 4.28	16.31± 8.33	7.90 ± 7.12	16.85± 6.27	5.70 ± 5.42	0.39 ± 0.75	6.54 ± 3.93	1.96 ± 2.08	0.02 ± 0.05	0.01 ± 0.04

6b *Palaemon*

Experiment	State (% of total time)								
	W	Wv	P	Pv	Z	R	G	S	L b
Control	17.52±3.40	1.94 ± 2.10	3.45± 1.44	0.93± 0.85	32.26±11.89	7.79± 9.02	16.38±8.17	19.74± 8.83	–
Curve	32.97±12.97	0.07 ± 0.06	2.40±3.06	2.71± 3.40	21.25±17.52	0.34± 0.35	0.70± 0.47	33.78±13.50	5.78± 5.03
Slope	46.05±11.20	0	1.65± 2.26	5.13± 5.92	14.77±14.11	0.12± 0.21	2.84± 2.05	27.49±10.76	1.94± 1.89

6c *Cherax albertisii*

Experiment	State (% of total time)										
	W	Wv	P	Pv	R	G	S	B	Lb	Push	Fall
Control	16.90±7.61	0.70±0.86	0.21±0.19	1.76±1.51	32.31±9.66	8.97±7.95	36.46±6.84	1.03±1.23	–	–	–
Curve	23.95±2.79	1.75±1.19	0.31±0.29	12.41±15.83	11.53±11.14	1.79±1.71	39.47±14.61	1.75±3.33	0.54±1.01	6.11±10.10	0.39±0.59
Slope	32.94±13.11	1.41±2.63	6.94±10.30*	6.06±6.58	7.56±7.78	2.05±2.95	35.47±13.66	2.05±4.16	3.63±3.39	1.62±3.13	0.27±0.53

6d *Procambarus clarkii*

Experiment	State (% of total time)									
	W	Wv	P	Pv	R	G	S	Lb	Push	Fall
Control	39.54± 0.02	1.13± 1.44*	0.60± 0.12	0.53±0.62	19.64± 3.51	2.44±2.87	36.12±2.84	–	–	–
Curve	10.15	41.74	4.27	0.49	5.02	0.05	34.77	0	3.02	0.49
Slope	51.69±13.81	1.20±1.08	0.18±0.45	0.94± 1.37	18.86±12.18	3.30± 8.08	19.99±2.89	1.17±2.46	2.54±3.43	0.13±0.21

6e *Homarus gammarus*

Experiment	State (% of total time)											
	W	Wv	P	Pv	R	G	S	W	F	Lb	Push	Turn
Control	22.91± 9.66	22.91±9.66	0.22±0.10	0.57± 0.35	25.33±14.92	10.52±8.00	22.91±2.93	15.05±14.25	0.82 ± 1.09	–	–	–
Curve	11.05± 5.55	0	0.46± 0.64	0	55.52±26.37	1.24± 0.28	11.50±3.93	14.22±13.40	0	0	2.34± 3.31	0
Slope	23.68±6.66	0	4.41± 3.20	0.42± 0.96	29.55±10.78	5.12± 6.57	22.34±11.05	6.13±9.97	1.61±3.27	0.83± 0.60	5.72 ± 1.60	0.18± 0.27

Table 7: Correlation test between the percentage of time spent on the different behavioural states and the time spent out of sight Of every state the correlation coefficient (R) is given. The correlation is significant if P < 0.05.

|		Palaemon	Cherax	Procambarus	Homarus
Walking	R	-0.280	-0.496	-0.846	0.150
	P	0.466	0.145	0.071	0.700
Vertical walking	R	-0.141	-0.414	-0.840	-0.041
	P	0.718	0.235	0.075	0.916
Walking with pleo.	R	-0.395	-0.294	-0.051	-0.293
	P	0.292	0.410	0.936	0.444
Vert. Walk with pleo.	R	-0.053	-0.260	-0.154	-0.025
	P	0.891	0.469	0.805	0.949
Resting	R	-0.127	-0.592	-0.233	-0.487
	P	0.744	0.072	0.706	0.184
Grooming	R	-0.001	0.374	0.724	-0.360
	P	0.998	0.287	0.167	0.342
Scanning	R	-0.067	-0.276	-0.796	-0.021
	P	0.864	0.441	0.107	-0.958
Bulldozing	R	X	-0.113	X	-0.141
	P	X	0.756	X	0.717
Swimming	R	-0.545	X	X	X
	P	0.129	X	X	X
Fanning	R	X	X	X	-0.236
	P	X	X	X	0.541

Weight of drying crayfishes
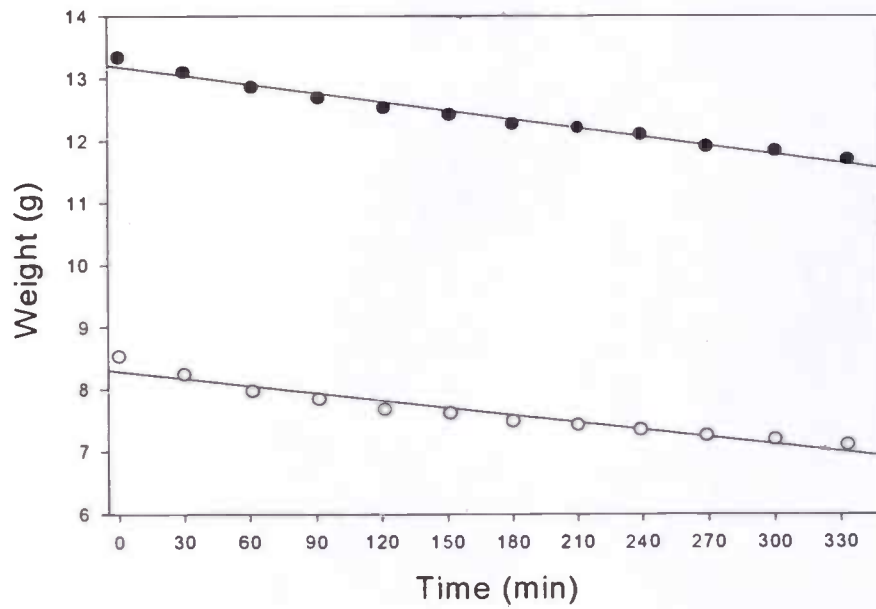
Fig. 12: The regression coefficients of both plots are higher than 0.95. The weight-loss wasn't significantly higher at the beginning of the experiment. After three hours the outside of the exoskeleton was completely dry.