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STUDIES ON THE BIOCHEMICAL VARIABILITY OF TWO NATURAL CRAYFISH POPULATIONS IN THE INTERMOLT STAGE

A. ZEKHNINI, C. DESVILETTES, C. CHAISEMARTIN

Université de Limoges, Faculté des Sciences, Laboratoire de biologie des populations, Hydrobiologie, 87060 Limoges cédex, France

ASTACIDAE
ÉCREVISSES
AUSTROPOTAMOBIUS PALLIPES
PACIFASTACUS LENIUSCULUS
STADE C4 D'INTERMUE
COMPOSITION BIOCHIMIQUE

RÉSUMÉ – La composition en lipides, glucides, et protéines au stade physiologique d'équilibre de l'intermue chez les deux espèces d'Ecrevisses sauvages, *A. pallipes* et *P. leniusculus*, occupant le même biotope, diffèrent significativement. Les lipides totaux révèlent des phospholipides et triglycérides en très grandes quantités par rapport aux acides gras libres et au cholestérol. Des différences dans le sexe et l'espèce sont notées dans la teneur en lipides, glucides, protéines et les différents composants lipidiques. La teneur en lipides, glucides, et protéines au stade C4 d'intermue, chez les deux espèces d'Ecrevisses, connait des variations significatives avec la taille.

ASTACIDAE
CRAYFISHS
AUSTROPOTAMOBIUS PALLIPES
PACIFASTACUS LENIUSCULUS
C4 STAGE OF INTERMOLT
BIOCHEMICAL COMPOSITION

ABSTRACT – In two crayfish species, *Austropotamobius pallipes* (Lereboullet, 1858) and *Pacifastacus leniusculus* (Dana, 1852), living in the same biotope, the lipid, protein and carbohydrate composition was significantly different at the physiological balance stage of intermolt. Total lipids contained high rates of phospholipids and triglycerids in comparison with sterols and esterified sterols. Sex and species differences in lipid, protein and carbohydrate rates and in the different lipid components are observed. Lipids, proteins and carbohydrates in the « C4 » stage of intermolt, in both species, showed significant variations with size.

INTRODUCTION

The recent focus on crayfish studies can easily be explained by new possibilities of crustacean farming, and also by restricted distribution of wild species in Europe.

If metabolism of biochemical components in *Decapoda* has been thoroughly studied (O'Connor and Gilbert, 1968; C. Chaisemartin, 1971, 1984) some questions are still unsolved.

In the Astacidae, the molt cycle (Chaisemartin, 1967), season and starvation (Chaisemartin, 1974) influence the metabolism of calcium, lipids and carbohydrates.

Therefore, we tried to compare the main metabolic components between *Austropotamobius pallipes* and *Pacifastacus leniusculus* at the physiological balance stage of intermolt. We also tried to analyse the change in components according to the size of crayfish.

STUDIED STREAMS AND METHODS

Both species of crayfish were caught in river Anglin, a little stream in Limousin (Middle West of France), 210 m above sea level.

The capture was done with a « Haveneau » fishing net. The length of each animal was routinely measured as the distance between the rostral apex and the telson. The fresh weight was also determined. We exposed the crayfish to 105°C during 4 hours to determine dry weight.

Lipids were extracted by chloroform methanol (2 : 1) according to Folch's method (Folch *et al.* 1957). Phospholipids were analysed by the colorimetric method (Barnes & Blackstock 1973) and triglycerids were estimated by the method of Bouletreau 1977. Protein concentrations were determined by using Lowry's method (Lowry *et al.* 1951) in which a sample of dry crayfish (0.1 g) is mixed with 1 ml of NaOH in

Table I. – Percentage of biochemical components as a function of dry weight (m = Tσm). n : number of crayfish; m : mean; σm : standard deviation; T = t 0.05 = value of Student coefficient for p = 0.05 and (n^{-1}) degree of freedom.

		n	Carbohydrates m	= Tσm	Lipids m	= Tσm	Proteins m	= Tσm
Austropotamobius	male	5	17,85	= 3.2	47.85	= 9.8	05.27	=1.2
pallipes	female	5	14.59	= 1.5	58.89	= 7.2	08.25	=1.27
Pacifastacus	male	5	10.05	= 1.8	49.70	= 8.0	12.85	=2.3
leniusculus	female	5	11.04	= 1.9	26.53	= 3.7	16.54	=4.4

a potter; after a short centrifugation (5 min at 1000 g), absorbance is read at 620 nm. Total carbohydrates were analysed by the glucose-oxydase-peroxydase method (Chaisemartin 1971; Murat & Serfaty 1974). Sterols were identified according to Moore & Baumann (1952) with a spectrophotometer.

The data of table I and II were presented as means. The confidence limits were calculated according to : C.L. = T 0.05 σm. σm is the standard deviation and T 0.05 the value of Student's coefficient for 0.05 probability and (n-1) degrees of freedom. The mean values were compared for significant differences using Student's t-test for small samples (n < 30).

RESULTS AND DISCUSSION

Table I shows the quantity of lipids, proteins and carbohydrates in the two wild species at the «C4» stage of intermolt (Drach & Tchernigovtzeff, 1967). The values in Figure 1 and 2 indicate that lipids are quantitatively the most important

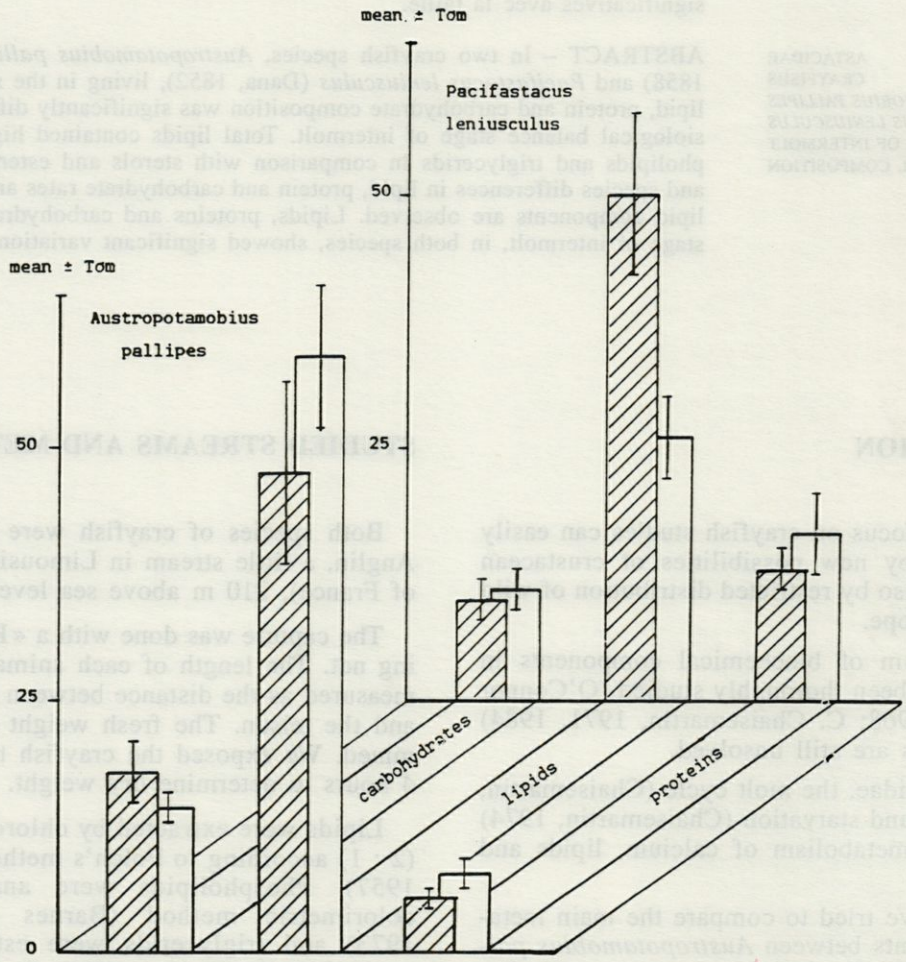

Fig. 1. – Percentage of major biochemical components in two crayfishs species. White : female; hatched part : male.

component. In *Artemia* (Nauplii larvae), percentages of biochemical parameters (according to dry weight) are divided up in the following way : (Barnes, 1973; Benyts *et al.* 1978).

Carbohydrates : 6 %

Fatty acids : 16 %

Proteins : 50 %

In *Cancer pagurus*, Giese (1966) found 2.3 % lipids and 22.4

% proteins. Chaisemartin *et al.* (1984) reported that *A. pallipes* fed with a percentage varying from 2.5 % to 4 % of body weight/day, had the following composition :

Carbohydrates : 11.9 to 17.8 %

Lipids : 66.2 to 17.3 %

Proteins : 10.4 to 15.1 %

These results, determined from the dry weight in stabulation conditions, are not very far from the ones found in wild animals.

Circulating biochemical components permit to distinguish crayfish populations. Thus Kabre & Chaisemartin (1987) discriminated two types of crayfish according to their organic haemolymph content. *A. pallipes* from a polluted area and *Orconectes limosus* contain more glucides and less lipids and proteins. These authors have concluded to the existence of a specific pattern of organic constituents in the haemolymph for each population.

Table II. – Major lipids components (% of Total lipids). m = Tom; m = mean; om : standard deviation; T : t 0.05 = value of Student coefficient for p = 0.05 and (n^{-1}) degree of freedom.

			Phospholipids		Triglycerids		Sterols		Esterified sterols	
		n	m	±Tom	m	±Tom	m	±Tom	m	±Tom
Austropotamobius pallipes	male	5	40.0	11.5	37.0	10.0	9.0	11.2	1.60	10.8
	female	5	50.5	11.3	40.0	11.3	12	11.4	7.52	10.6
Pacifastacus leniusculus	male	5	50.5	10.0	29.3	12.6	5.97	10.5	3.90	11.5
	female	5	53.6	10.0	27.6	11.0	0.46	10.8	3.50	10.5

Carbohydrate, lipid and protein values show large variations (Fig. 1). These variations are connected with physiological differences in the two species studied. Similar observations were reported by Chaisemartin (1971) in three other species of crayfish.

Wild crayfish develop variable biochemical concentrations according to the season of measurements and the type of food (Kabre, 1983).

We found intraspecific variations in the percentage of total lipids (according to dry weight) in the two wild species. Males of *P. leniusculus* had a total lipid percentage twice that of the females, but the opposite situation was shown by *A. pallipes*.

In each species there were important differences in metabolic components according to size. Males of *A. pallipes* showed a high decrease of total lipids at a size of 60 mm, the same happened in females of *P. leniusculus* when they reched a size of 90 mm. On the other hand, figure 3 shows quite a linear increase of total lipids in *A. pallipes* females.

At the intermolt stage (C4) lipid categories were in different quantities depending on the sex. Phospholipids, structural material, showed very similar data in the species (Fig. 2). We observed

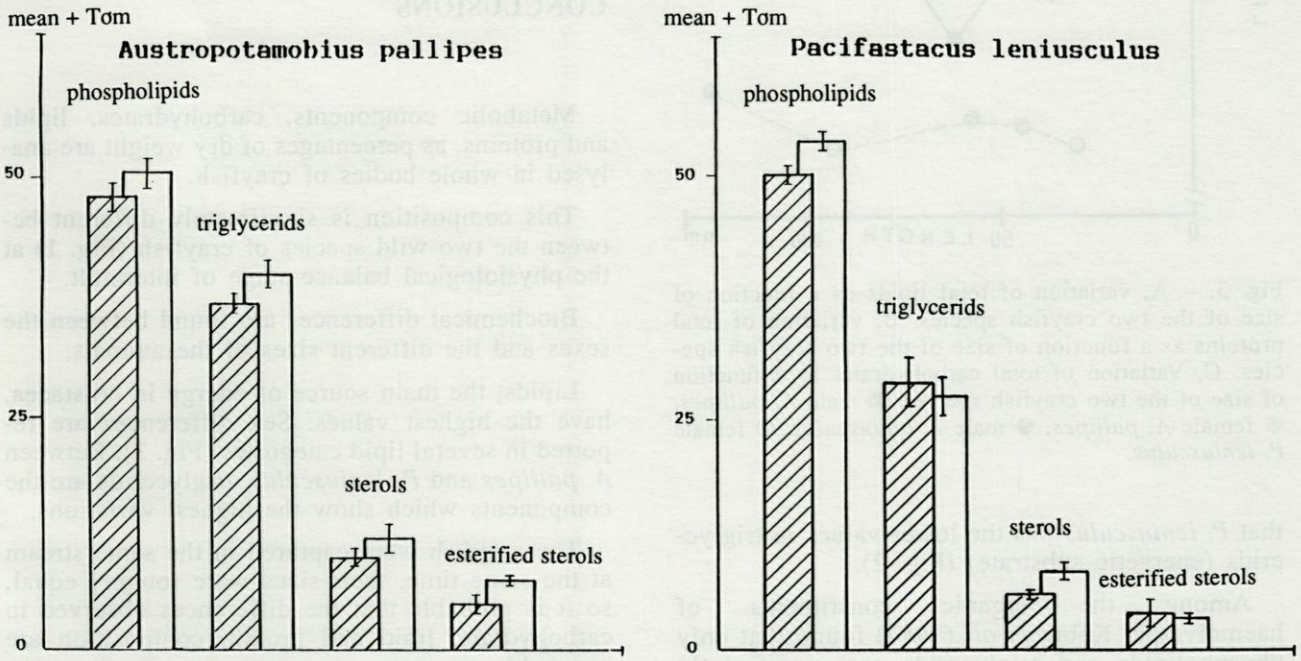

Fig. 2. – Major lipid components (% of total lipids). Hatched part : male; white : female.

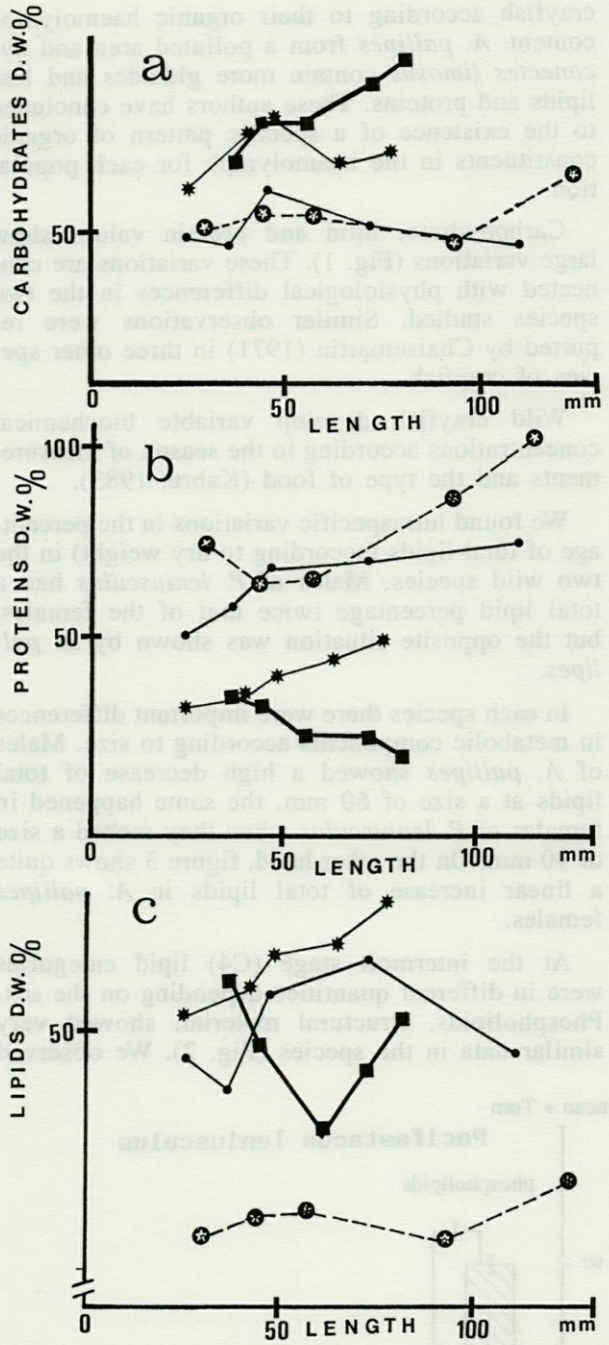

Fig. 3. – A, variation of total lipids as a function of size of the two crayfish species. B, variation of total proteins as a fonction of size of the two crayfish species. C, Variation of total carbohydrates as a function of size of the two crayfish species. ■ male *A. pallipes*, ✱ female *A. pallipes*, ● male *P. leniusculus*, ✪ female *P. leniusculus*.

that *P. leniusculus* has the lower values in triglycerids (energetic substrate) (Fig. 2).

Among the organic constituents of haemolymph, Kabre *et al.* (1987) found that only phospholipids and triglycerids can significantly differenciate healthy populations of A. *pallipes* from populations parasited by *Thelohania contejeani* (microsporidian). In our study, two other components also distinguish populations : sterols and esterified sterols (Fig. 2).

Total carbohydrates showed virtually identical values in both sexes of a same species, with slightly higher amounts in *A. pallipes* (Fig. 1). Circulating carbohydrates increase under the influence of capture, anoxia, digestion, repetitive bleeding (Chaisemartin, 1971). According to Telford & Canard (1968), glucide increase is a stress response in invertebrates. In both species, total carbohydrates reach a maximum at a size of 50 mm (Fig. 3); above that limit these components become stabilized in *P. leniusculus* and raise in *A. pallipes* (Fig. 1).

Total proteins give, as a physiological « sign », an illustration of the disturbed state of a population (Chaisemartin, 1979; Kabre *et al.*, 1984). On the other hand, proteins do not give us possibilities of differentiating the population infected by *T. contejeani* (Kabre G., 1987). Table I and Fig. 1 indicate significant differences in protein data between the two species. As a function of the size, percentage of total proteins increases significately during the intermolt, but not in the case of *A. pallipes* males. These show a drecrease of total proteins (Fig. 3). Among metabolic components, total proteins are used at least as energetic substrate in Crustacea (C. Chaisemartin, 1971). It seems that *A. pallipes* use proteins as energetic substrate before carbohydrates and lipids (Fig. 1).

CONCLUSIONS

Metabolic components, carbohydrates, lipids and proteins, as percentages of dry weight are analysed in whole bodies of crayfish.

This composition is significately different between the two wild species of crayfish (Fig. 1) at the physiological balance stage of intermolt.

Biochemical differences are found between the sexes and the different sizes of the animals.

Lipids, the main source of energy in crustacea, have the highest values. Sex differences are reported in several lipid categories (Fig. 3). Between *A. pallipes* and *P. leniusculus*, triglycerids are the components which show the highest variations.

The crayfish were captured in the same stream at the same time, their sizes were roughly equal, so it is probable that the differences observed in carbohydrate, lipid and protein composition are ascribable to some divergent as : Feeding behaviour, Metabolic activity, Genetic factors ...

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