Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part A 147 (2007) 1015–1027
CBP
www.elsevier.com/locate/cbpa

Seasonal variability of free amino acids in two marine bivalves, *Macoma balthica* and *Mytilus* spp., in relation to environmental and physiological factors

S. Kube [a,*], A. Sokolowski [b], J.M. Jansen [c], D. Schiedek [a]

[a] *Baltic Sea Research Institute Warnemuende, Seestrasse 15, 18119 Rostock, Germany*
[b] *University of Gdansk, Institute of Oceanography, Al. Pilsudskiego 46, 81-378 Gdynia, Poland*
[c] *Netherlands Institute of Ecology, Centre for Estuarine and Marine Ecology, Korringaweg 7, 4401 Yerseke, The Netherlands*

Received 2 August 2006; received in revised form 9 March 2007; accepted 10 March 2007
Available online 16 March 2007

Abstract

The seasonal variability of the intracellular free amino acid (FAA) concentration was studied in 5 *Macoma balthica* populations and 7 *Mytilus* spp. populations along their European distribution. Because of the well known physiological role of FAA as organic osmolytes for salinity induced cell volume regulation in marine osmoconformers, FAA variations were compared in bivalve populations that were exposed to high vs. low intraannual salinity fluctuations. In general, seasonal FAA variations were more pronounced in *M. balthica* than in *Mytilus* spp. In both bivalve taxa from different locations in the Baltic Sea, highest FAA concentrations were found in autumn and winter and low FAA concentrations were measured in summer. Seasonal patterns were less pronounced in both taxa at locations with constant salinity conditions. In contrast to Baltic Sea populations, Atlantic and Mediterranean bivalves showed high FAA concentrations in summer and low values in winter, regardless of seasonal salinity fluctuations. Significant seasonal FAA variations at locations with constant salinity conditions showed that salinity appeared not to be the main factor in determining FAA concentrations. The seasonal patterns of the main FAA pool components, i.e. alanine, glycine and taurine, are discussed in the context of seasonal variations in environmental factors (salinity, temperature) and physiological state (glycogen content, reproductive stage).

Keywords: Bivalve; Osmoconformer; Free amino acids; Salinity; Temperature; Glycogen; Gonadal index

1. Introduction

Free amino acids (FAA), including the non-proteic 2-aminoethanesulfonic acid taurine, represent 6 to 11% of the total tissue dry mass in marine invertebrates, reaching concentrations of up to 400 mm (Zandee et al., 1980; Bishop et al., 1983). Among the FAA, alanine, glycine and taurine are most abundant in marine molluscs (Baginski and Pierce, 1977; Shumway et al., 1977; Deaton et al., 1985; De Vooys, 1991; Matsushima and Hayashi, 1992; Pierce et al., 1992), polychaetes (Koenig et al., 1981; Hülsmann et al., 1991; Blank et al., 2004), crustaceans (Goolish and Burton, 1989; Bishop and Burton, 1993), and other marine invertebrates (e.g. sipunculids — Peng et al., 1994).

Several experimental studies have shown that FAA are involved in a variety of metabolic processes in marine invertebrates (Bishop et al., 1983). Fluctuations of the osmotic pressure in the external medium are known to be a prominent factor causing alterations in FAA concentrations. Osmoconformers utilise intracellular FAA as important organic osmolytes to minimize the cellular osmotic pressure, thereby preventing cells from shrinkage or swelling if the osmotic pressure of the ambient environment changes (Gilles, 1987; Yancey, 2005). The up- and down-regulation of the amino acids alanine, glycine, arginine, aspartate, glutamate, and taurine has shown to be particularly important in the process of isoosmotic cell volume regulation (Bayne et al., 1976; Baginski and Pierce, 1977; Shumway et al., 1977; Livingstone et al., 1979). Large seasonal temperature fluctuations and associated changes in oxygen availability (related to high water temperatures or ice coverage) can also

* Corresponding author. Tel.: +49 381 5197 205; fax: +49 381 5197 211.
E-mail address: sandra.kube@uni-rostock.de (S. Kube).

1095-6433/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2007.03.012

affect FAA concentrations due to induction of anaerobiotic metabolism (Zurburg and De Zwaan, 1981; Powell et al., 1982). At least three amino acids are involved in anaerobic metabolism. With the onset of anaerobiosis alanine accumulates as a product of the glutamate-pyruvate transamination with aspartate being the amino group donor for α-ketoglutatrate to form glutamate and oxaloacetate (de Zwaan, 1991).

FAA concentrations in aquatic invertebrates depend not exclusively on environmental conditions. They also vary with the developmental and physiological conditions of the animals Helland et al., 2000; Chiou et al., 2001; Rosa et al., 2005). They are known to serve, besides reserve constituents such as glycogen, lipid and protein, as energy sources during reproduction, growth, and hypoxic conditions (Gäde, 1988). Furthermore, glycine has been reported to fluctuate significantly with the reproductive cycle in marine invertebrates (Kasshau and McCommas, 1982; Zurburg et al., 1989; Sokolowski et al., 2003).

Hence, the quantitative composition of the FAA pool in marine invertebrates reflects the interaction of several synergistic as well as antagonistic factors and shows, therefore, not only species specific differences but also spatial variations within species (Zurburg et al., 1989; Pierce et al., 1992). Based on a large scale comparison along the European coast clear spatial differences in the FAA pool have also been shown for two dominant bivalve taxa, *Mytilus* spp. and *Macoma balthica* (Kube et al., 2006).

However, little is known about the natural variability of the FAA pool within a species across large spatial and temporal gradients (Zurburg at al., 1989; Hummel et al., 1994). The present study aims to show the range of the intra- and interspecific seasonal variability of FAA in *Mytilus* spp. and *M. balthica* from eight shallow water habitats along their western European distribution. We compare seasonal FAA variations in bivalve populations that are exposed to high vs. low intraannual salinity fluctuations. The patterns of the main FAA pool components, i.e. alanine, glycine and taurine, are discussed in the context of seasonal changes in environmental factors (salinity, temperature) and physiological state (glycogen content, reproductive stage).

2. Materials and methods

2.1. Sampling locations

Mytilus spp. and *M. balthica* were collected between July 2003 and July 2004 at eight locations along the European coast (Table 1, Fig. 1). Salinity and water temperature were measured at each location and each sampling date while sampling (Fig. 2). In addition, hydrological monitoring data from different national sources were used from comparable sites close to the sampling stations (Table 1, Fig. 1). In general, both data sets show similar trends. Remarkable deviations, however, occurred when comparing own measurements with those from the national monitoring data obtained for the Westerschelde and the Bight of Aiguillon. This is most likely explained due to sampling of these estuaries during low tide, when salinity and temperature in the tidal creeks vary strongly. For the Bidasoa estuary, continuous monitoring data were not available. In our study salinity is given in PSU (practical salinity units) since this unit is used in the hydrological databases.

Two sampling sites in the northern Baltic Sea (Bothnian Gulf: 63°39′N, 19°50′E; Stockholm archipelago (Askö): 58°50′N, 17°38′E) were characterized by seasonally constant low salinity conditions (<7 PSU) and temperatures ranging between 0 °C and 20 °C. In the southern Baltic Sea (Mecklenburg Bight, 54°01′N, 11°29′E) salinity fluctuated stochastically between 11 and 18 PSU due to saline water inflows from the Kattegat into the Baltic proper and temperature ranged between 2 °C and 22 °C. In the Baltic Sea, bivalves were sampled in shallow water (<1 m). Bivalves from three Atlantic estuaries were collected at intertidal stations during low tide at low shore: Westerschelde estuary (51°23′N, 3°38′E), Bight of Aiguillon (46°16′N, 1°12′W) and Bidasoa estuary (43°22′N,

Table 1
Seasonal sampling of *Macoma balthica* and *Mytilus* spp. from April 2003 to July 2004 (sampling date and sample size)

Location		Sampled species	Sampling date and sample size						Source of hydrological data
			April 2003	July 2003	October 2003	January 2004	April 2004	July 2004	
Bothnian Gulf	BG	*M. balthica*		10	14	10	10	11	SMHI's Swedish Ocean Archive SHARK (site B7), www.smhi.se
Askö	ASK	*M. trossulus*		10	8	10	9	7	SMHI's Swedish Ocean Archive
		M. balthica		10	8	10	20	10	SHARK (site B1), www.smhi.se
Mecklenburg Bight	MB	*M. edulis*		10	10	7	10		Landesamt für Umwelt, Naturschutz und
		M. balthica	10	10	10	10	10	8	Geologie Mecklenburg-Vorpommern (site WB6)
Grevelingen	GRE	*M. edulis*		10	10	10	5	9	www.waterbase.nl (site "Dreischor")
Westerschelde	WES	*M. edulis*		10	10	10	10	9	www.waterbase.nl (site "Hansweert geul")
		M. balthica		9	9	9	9	18	
Bight of Aiguillon	BA	*M. edulis*		10	10	10	8	10	Institut français de recherche pour l'exploitation
		M. balthica		10	10	10	20	10	de la mer (site "La Carrelere"), www.ifremer.fr
Bidasoa	BID	*M. galloprovincialis*		10	10	10	9	9	This study
Marseille	MAR	*M. galloprovincialis*		10	3	10	10	9	Laboratoire d'Océanographie et de Biogéochimie, Marseille (site "Frioul")

The last column gives the source of hydrological data and the corresponding salinity and temperature measurement sites, which were used in Figs. 1 and 2 and for temperature and salinity correlations (Tables 2 and 3).

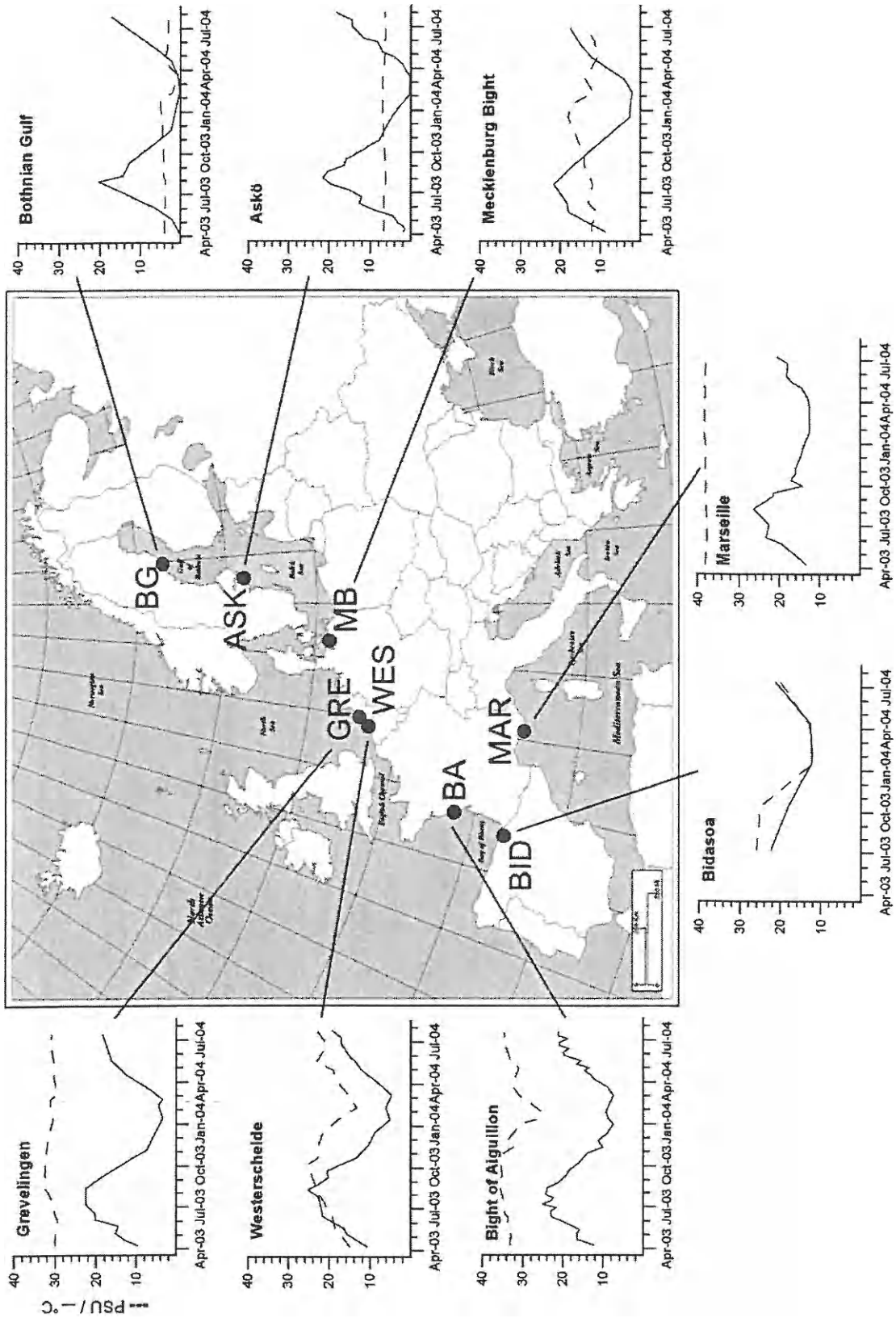

Fig. 1. Field sampling locations, monthly means of water temperature (solid line) and salinity (dashed line) during the sampling period. Temperature and salinity data were obtained from: SMHI's Swedish Ocean Archive SHARK, www.smhi.se (BG, ASK); Landesamt für Umwelt, Naturschutz und Geologie Mecklenburg-Vorpommern (MB); www.waterbase.nl (WES, GRE); Institut français de recherche pour l'exploitation de la mer, www.ifremer.fr (BA); this study (BID), Laboratoire d'Océanographie et de Biogéochimie, Centre d'Océanologie de Marseille (MAR).

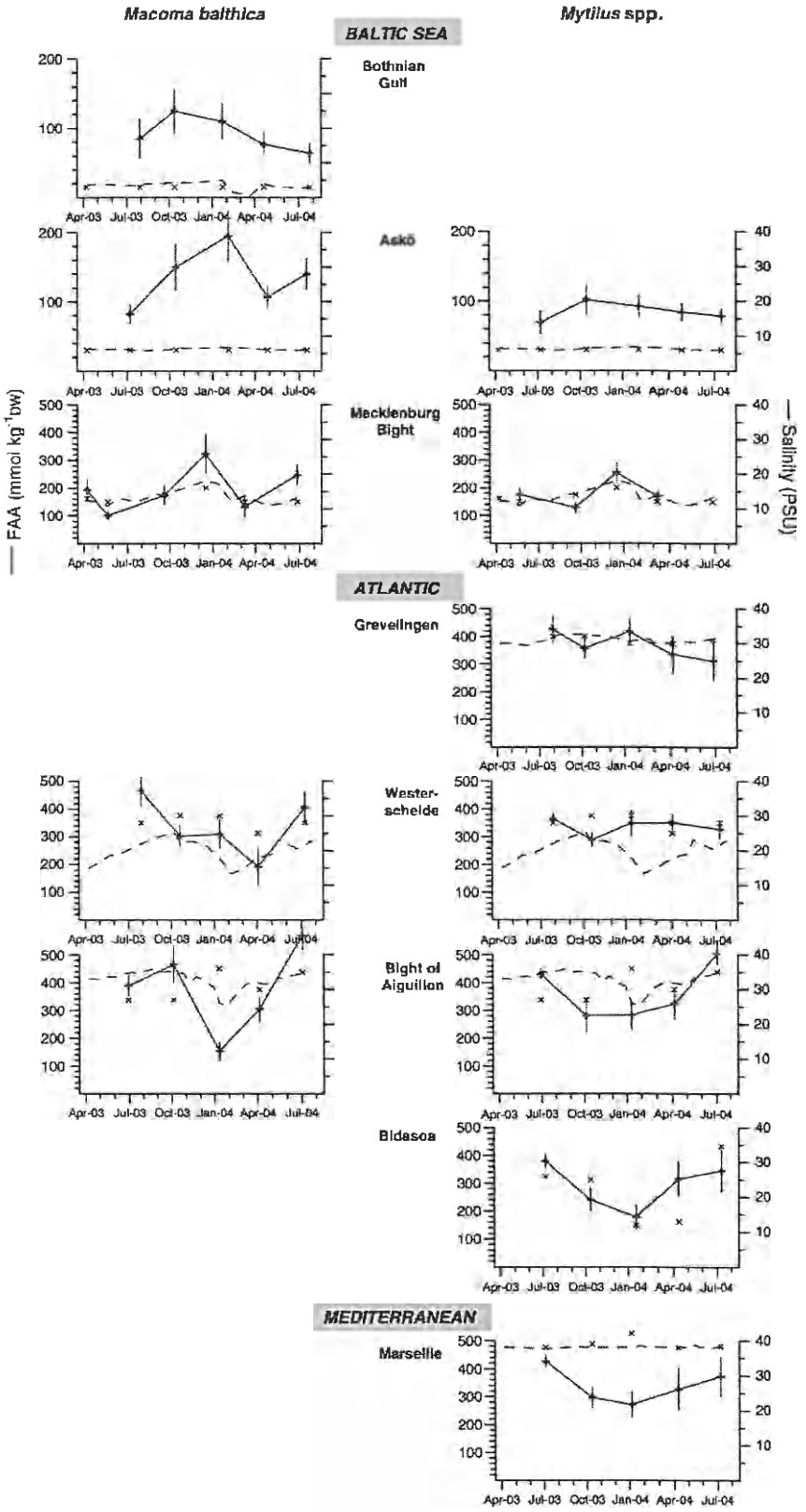

Fig. 2. Seasonal fluctuations of the total FAA concentration (sum of taurine, glycine, alanine, aspartate, glutamate, arginine, leucine, proline, valine) in *M. balthica* and *Mytilus* spp. at 8 locations along the European coastline (continuous line, mean mmol $kg^{-1}{}_{DW} \pm SD$). Salinity data (dashed line) were obtained from different monitoring programs (see Table 1) and by own measurements ("×").

1°46′W). The Atlantic estuaries were characterized by strong seasonal salinity fluctuations with lowest values in winter and higher values in summer. The range of salinity fluctuations during the study period was 14–27 PSU in the Westerschelde, 25–35 PSU in Bight of Aiguillon and 12–26 PSU in Bidasoa. The fourth Atlantic location was lake Grevelingen (51°44′N, 3°59′E), with constant salinity conditions throughout the year (30–32 PSU) and no tidal cycle. Seasonal temperature variations were similar at all four Atlantic sites (5 °C–25 °C). A Mediterranean sampling location was situated near Marseille (43°17′N, 05°21′E, sampling depth about 1 m). Here, the salinity was constant throughout the year (38–39 PSU) and the temperature ranged between 12 °C and 26 °C.

At each location and each sampling date, 3–10 *Mytilus* spp. with a shell length of 30–40 mm were collected by gently removing them from the hard substrate (Table 1). Eight to twenty *M. balthica* (shell length 10–15 mm) were sieved out from the sediment at each location (Table 1). All bivalves were transported frozen at −20 °C (3–5 days) and afterwards stored at −80 °C until dissection. Additionally, 10 specimens from each taxon and population were collected and stored in ethanol for the determination of the reproductive stage.

2.2. Biochemical analysis

The soft tissue of 3–20 specimens of *Mytilus* or *Macoma* from each location and each sampling date was dissected, individually freeze-dried and weighed. The dry tissue was ground to fine powder. 20–25 mg dry tissue powder were transferred to a 5-fold volume of ice-cold 0.6 M perchloric acid and homogenized.

100 µL of homogenized tissue of each individual were taken for quantitative enzymatic analysis of the glycogen content according to Keppler and Decker (1984). For the spectrophotometrically determination of NADPH as glucose equivalent at 339 nm, a 96 well plate reader (GENios, Tecan) was used. The glycogen content was calculated as percentage of tissue dry weight.

The remaining volume of homogenate was centrifuged at 12000 ×*g* for 20 min. The supernatant was taken for analysis of dansyl-derivatised free amino acids by HPLC (for details see Kube et al., 2006). External standards were used to identify and calculate the concentration of eight amino acids (glycine, alanine, aspartate, glutamate, arginine, leucine, proline, valine) and the 2-aminoethanesulfonic acid taurine (MERCK-Hitachi D-7000 software). Amino acid concentrations are expressed as mmol $kg^{-1}{}_{DW}$.

2.3. Reproductive stage

Ten individuals of *M. balthica* and *Mytilus* spp. were used to determine the gonadal index. Five developmental stages were distinguished by macro- and microscopically observation of the gonad and mantle tissue: (1) immature, transparent gonad tissue visible only on the posterior part of visceral mass above the foot, small gametes (spermatogonies and oogonies) closely connected with follicle epithelium, (2) developing, about 50% of visceral mass covered by white or transparent gonad tissue, follicle ducts branch from central to external part of gonad, numerous spermatocytes and oocytes; mature gametes account for less than 50% of all gametes; clearly visible follicles; gametes loosely arranged in follicles, (3) ripe, the whole surface covered by white compact gonad tissue; follicles have easily breaking wall, spermatozoa and ova close-packed in follicles; nuclei of mature gametes clearly visible, (4) spawning, gonads form swollen mass covering the whole surface of visceral mass; follicles filled with gametes, some follicles are open and empty, visible as fragments of follicle wall; appearance of spermatogonies and oogonies, (5) after spawning (resting), no traces of sexuality in the mantle; single mature gametes still remain in follicles; numerous fragments of follicle wall Caddy, 1967; Keck et al., 1975; Wenne, 1985). The gonadal index was calculated after Chipperfield (1953).

2.4. Statistics

The amplitude of seasonal variations of FAA was described as the maximum/minimum FAA concentration ratio at each

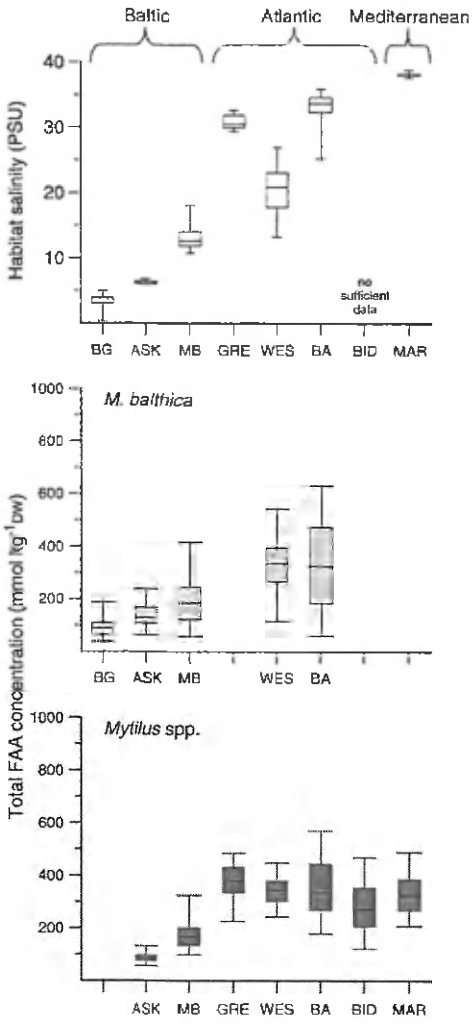

Fig. 3. Annual range of habitat salinity (above) and total FAA concentrations in *M. balthica* (middle) and *Mytilus* spp. (below) at 8 locations along the European coastline.

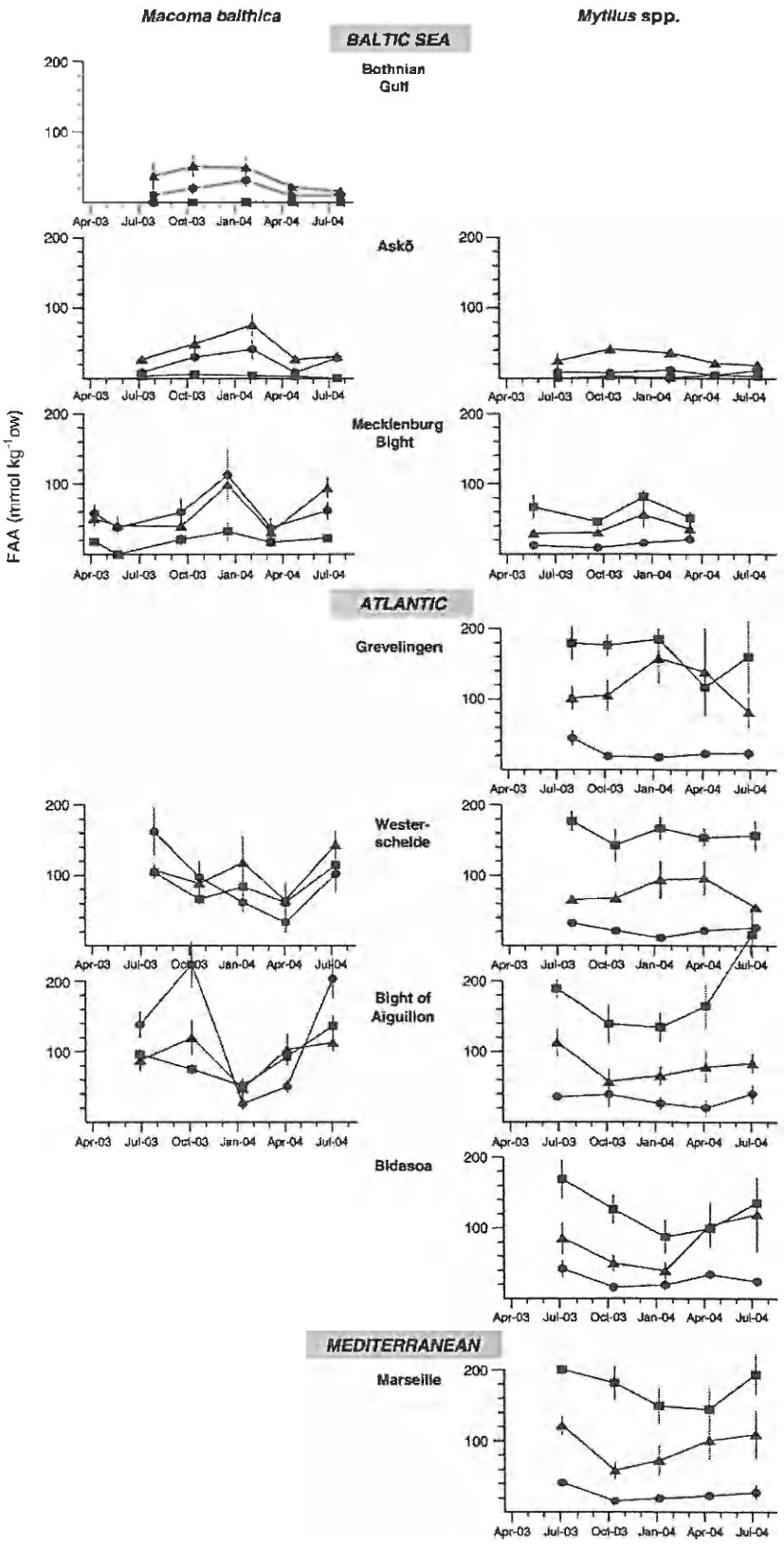

Fig. 4. Seasonal fluctuations of ● alanine, ▲ glycine and ■ taurine in *M. balthica* and *Mytilus* spp. at 8 locations along the European coastline (mean mmol kg^{-1}_{DW}±SD).

Table 2
Macoma balthica: determination coefficients (R^2) and levels of significance (*$p<0.05$, ***$p<0.001$) for the linear regression of free amino acids and salinity, temperature and glycogen content

		N	Salinity	Temperature	Glycogen
Alanine	BG	55		− 0.282***	− 0.004
	ASK	51		− 0.116*	− 0.000
	MB	43	0.544***	− 0.119*	0.007
	WES	48	0.391***	0.611***	0.553***
	BA	55	0.686***	0.680***	0.527***
Glycine	BG	55		− 0.073*	− 0.000
	ASK	51		− 0.230***	− 0.005
	MB	43	0.305***	− 0.020	0.000
	WES	48	0.054	0.076	0.004
	BA	55	0.534	0.232***	0.329***
Taurine	BG	55		− 0.042	− 0.004
	ASK	51		− 0.052	0.308***
	MB	43	0.509***	− 0.149*	− 0.000
	WES	48	0.096*	0.272***	0.025
	BA	55	0.335***	0.419***	0.030

For abbreviations of locations see Table 1.

location ("seasonal factor", Sokolowski et al., 2003). Linear regression analyses were performed for the relation of alanine, glycine and taurine with habitat salinity, temperature and glycogen. Since habitat salinity variations were extremely low in the Bothnian Gulf, at Askö and in Marseille throughout the study period, no regression analyses for salinity and FAA were performed for bivalves from these locations. One-way ANOVA and Scheffé post hoc tests were performed for the seasonal variability of the total sum and individual free amino acids. Statistical analyses were performed by using STATISTICA® StatSoft.

3. Results

The sum of all measured FAA changed significantly with season in all three Baltic populations of *M. balthica* and *Mytilus trossulus* and *Mytilus edulis*: highest FAA concentrations were measured in autumn and winter, lower concentrations were measured in spring and summer (Fig. 2). The FAA variability was lower in both taxa at locations with constant salinity conditions (ANOVA, *Macoma*: Bothnian Gulf $F_{4,50}=12.02$, $p<0.001$; Askö $F_{4,47}=14.3$, $p<0.001$; *Mytilus*: Askö $F_{4,34}=4.21$; $p<0.01$) compared to bivalves exposed to intraannual salinity fluctuations of about 8 PSU in Mecklenburg Bight (ANOVA, *Macoma*: $F_{4,38}=33.21$, $p<0.001$, *Mytilus*: $F_{3,28}=35.38$, $p<0.001$; Fig. 3).

Intraannual FAA concentrations in *M. balthica* from the Atlantic estuaries showed an opposite pattern compared to the Baltic Sea populations: high FAA concentrations in summer and low FAA concentrations in winter (Fig. 2). The seasonal variability of the total FAA concentration was higher in *M. balthica* from Bight of Aiguillon ($F_{4,50}=89.66$, $p<0.001$) than in *M. balthica* from Westerschelde ($F_{4,44}=26.9$, $p<0.001$, Fig. 3). The FAA concentrations in *M. edulis* from the Westerschelde estuary and lake Grevelingen fluctuated only little throughout the year (Westerschelde $F_{4,39}=9.54$, $p<0.001$, Grevelingen $F_{4,35}=7.03$, $p<0.001$). *M. edulis* from Bight of Aiguillon showed similar seasonal FAA patterns as *M. balthica* from the same location, but with a lower variance (Bight of Aiguillon $F_{4,38}=36.65$, $p<0.001$, Fig. 3).

The FAA concentration in *Mytilus galloprovincialis* from the Bidasoa estuary and Marseille did also drop in autumn and winter (Fig. 2) similar to the pattern found in *M. edulis* from the Bight of Aiguillon (ANOVA: Bidasoa $F_{4,38}=19.13$, $p<0.001$; Marseille $F_{4,37}=8.13$, $p<0.001$).

The dominant FAA in both bivalve taxa were alanine, glycine and taurine, which constituted together about 50–70% of the total FAA pool in the *Baltic* populations and about 80–90% in the Atlantic and Mediterranean populations.

In *M. balthica*, alanine, glycine and taurine showed similar seasonal cycles within populations with the exception that taurine was absent or extremely low concentrated in the northern Baltic Sea. In general, seasonal cycles of the three main FAA followed the typically opposite pattern in Baltic and Atlantic populations as described for the total FAA pool (Fig. 4). In Baltic Sea populations, all three FAA reached maximum values in winter, whereas in Atlantic populations highest concentrations were measured in summer. The amplitude of intraannual FAA variations (expressed as "seasonal factor") was highest for alanine (Baltic: 3.0–4.8, Atlantic: 4.9–8.6), less pronounced for glycine (Baltic: 2.8–3.2, Atlantic: 2.2–3.2) and very low for taurine (Baltic: 1.0–1.9, Atlantic: 1.9–2.7).

In *Mytilus* spp., the alanine, glycine and taurine concentrations fluctuated less throughout the year (Fig. 4). The amplitude of variation was similar for all three FAA (alanine: 2.0–3.2, glycine: 1.5–3.0, taurine: 1.5–2.0). Alanine concentrations in *Mytilus* spp. did not follow a seasonal trend in Baltic *Mytilus* populations, but showed generally higher levels in summer than

Table 3
Mytilus spp.: determination coefficients (R^2) and levels of significance (*$p<0.05$, **$p<0.01$, ***$p<0.001$) for the linear regression of free amino acids and salinity, temperature and glycogen content

		N	Salinity	Temperature	Glycogen
Alanine	ASK	39		<0.001	− 0.001
	MB	32	− 0.017	− 0.488***	− 0.038
	GRE	40		0.493***	0.106*
	WES	44	0.200**	0.552***	0.742***
	BA	43	0.165**	0.233**	0.328***
	BID	42	<0.001	0.015	0.261***
	MAR	42		0.309***	0.705***
Glycine	ASK	39		− 0.089	− 0.013
	MB	32	0.370***	− 0.291**	0.089
	GRE	40		− 0.322***	− 0.182**
	WES	44	− 0.367***	− 0.385***	− 0.326***
	BA	43	0.037	0.151**	− 0.093
	BID	42	0.001	0.091*	0.019
	MAR	42		0.042	0.158*
Taurine	ASK	39		− 0.026	0.068
	MB	32	0.237**	− 0.117	0.050
	GRE	40		0.015	− 0.005
	WES	44	0.022	0.099*	0.083
	BA	43	0.162**	0.328***	− <0.001
	BID	42	0.478*	0.566***	0.274***
	MAR	42		0.471***	0.463***

For abbreviations of locations see Table 1.

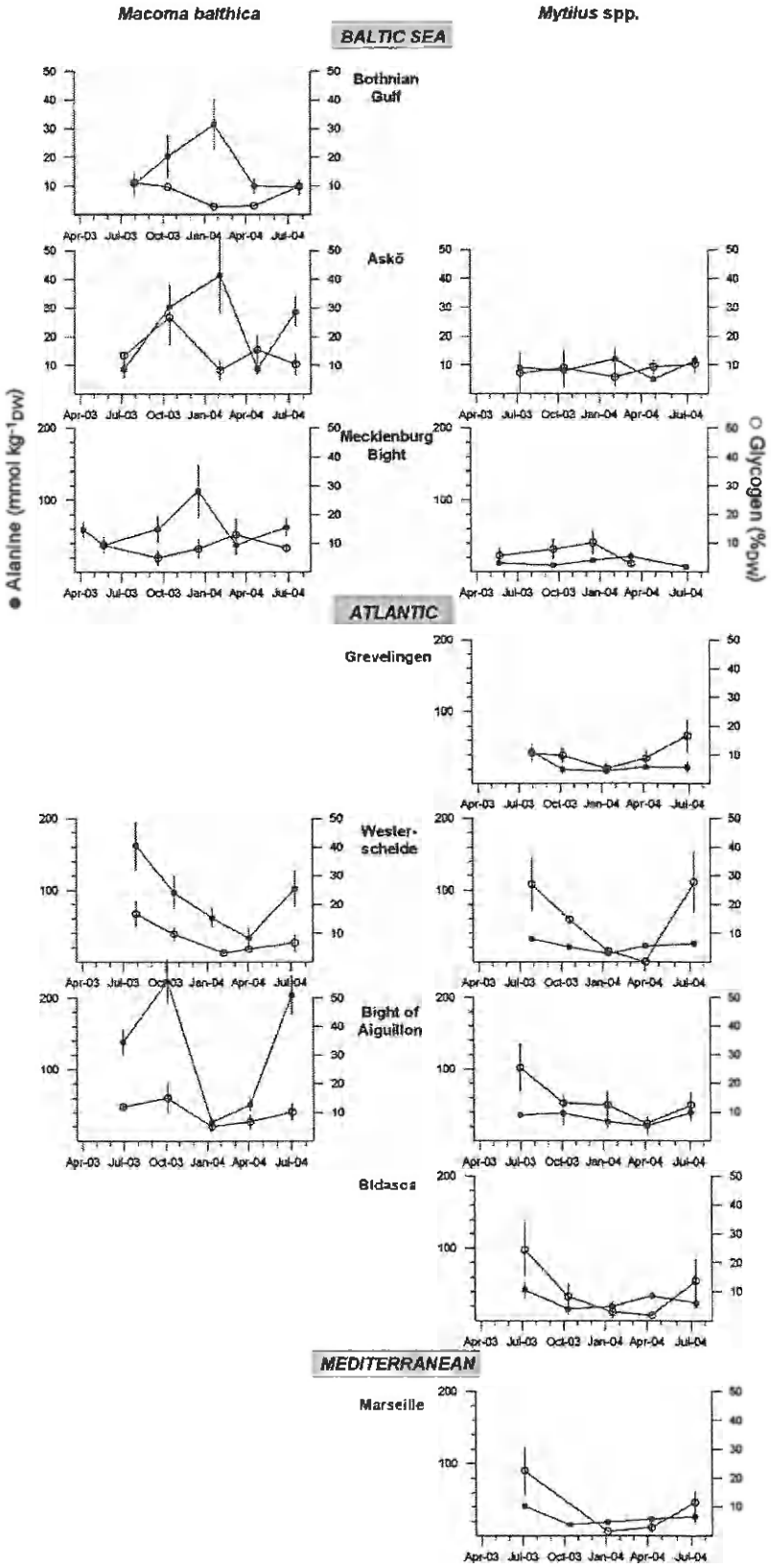

Fig. 5. Seasonal fluctuations of ● alanine (mean mmol $kg^{-1}_{DW} \pm SD$) and ○ glycogen (mean % of tissue DW ± SD) in *M. balthica* and *Mytilus* spp. at 8 locations along the European coastline.

in winter in Atlantic and Mediterranean populations (Fig. 4). Seasonal glycine and taurine fluctuations in the Baltic Sea *Mytilus* populations were very similar as described for *M. balthica*: high concentrations were recorded in winter, low concentrations in spring and summer. The intraannual pattern and amplitude of glycine in *M. edulis* from Grevelingen and Westerschelde was similar to the Baltic populations (seasonal factor 1.9 and 1.5, respectively). In contrast, the more southern Atlantic (Bight of Aiguiilon, Bidasoa) and Mediterranean mussels (Marseille) showed a decrease of glycine and taurine in winter (Fig. 4).

To explain the observed seasonal patterns of alanine, glycine and taurine the values were related to environmental factors (salinity, temperature) and physiological parameters (glycogen content, gonadal index).

In all studied *M. balthica* populations that were exposed to intraannual salinity fluctuations (Mecklenburg Bight, Westerschelde, Bight of Aiguillon), the three main FAA were positively correlated with the habitat salinity. These correlations were significant for alanine and taurine and less pronounced for glycine (Table 2). Furthermore, alanine and glycine were negatively correlated with habitat temperature in all Baltic populations. These relations were stronger in *M. balthica* from the Bothnian Gulf and Askö than in those from Mecklenburg Bight. In Atlantic populations all three FAA correlated positively with habitat temperature (Table 2).

In *Mytilus* spp., habitat salinity appeared not to be the main factor forcing the concentrations of the most abundant FAA (Table 3). Concerning alanine, positive correlations with habitat salinity were only observed in mussels from Westerschelde and Bight of Aiguillon. No such trend was found concerning the impact of salinity on intraannual glycine variations. Only taurine showed a weak positive correlation with the habitat salinity in all populations. The relation of FAA with temperature in *Mytilus* spp. generally showed the same pattern as in *M. balthica* (Tables 2 and 3). All three FAA were not, or negatively correlated with habitat temperature in the Baltic populations and positively related with temperature in the Atlantic and Mediterranean populations. One remarkable exception from this pattern showed glycine in *M. edulis* from Grevelingen and Westerschelde where, similarly to the southern Baltic Sea, a negative glycine-temperature correlation was found.

The observed variations of glycogen showed a characteristic seasonal pattern in both taxa: high values were found in summer and autumn, low values in winter and spring. The values ranged between 2 and 20% of the tissue dry weight (Fig. 5). When

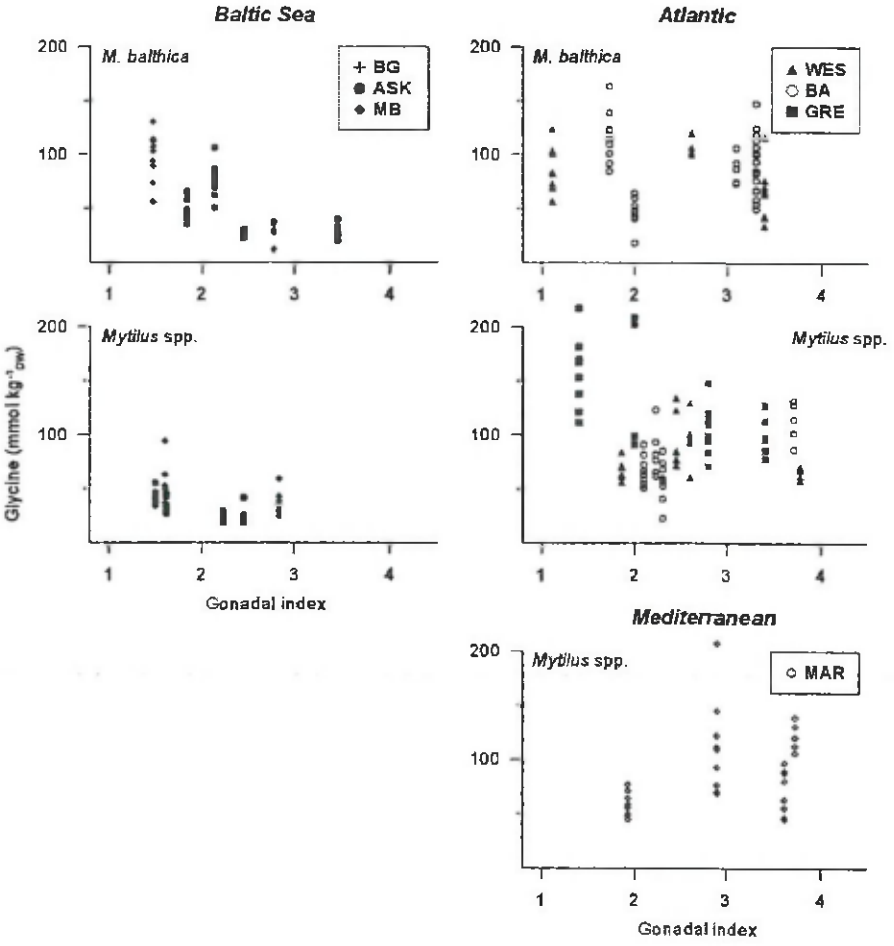

Fig. 6. The relation between glycine concentration and the gonadal index of *M. balthica* and *Mytilus* spp. at 8 locations. For abbreviations of locations see Table 1.

relating the tissue glycogen content to the FAA concentrations, significant relations were revealed for alanine (Tables 2 and 3). The alanine concentration was positively related to the glycogen reserves in *M. balthica* and *Mytilus* spp. from the Atlantic and Mediterranean populations. In contrast, no relation between alanine and glycogen was observed in *M. balthica* and *Mytilus* spp. from the Baltic Sea (Tables 2 and 3). Especially in the most northern *M. balthica* populations an inverse trend of alanine and glycogen was observed in winter: the alanine content increased while the glycogen content declined (Fig. 5).

The glycine concentration appeared to decrease with gonadal development until spawning in both bivalve taxa in all Baltic Sea populations, as well as in *M. edulis* from Grevelingen (Fig. 6). In all other Atlantic and in Mediterranean bivalves, glycine did not show any relation to the reproductive stage (Fig. 6).

4. Discussion

Free amino acids serve as metabolic compatible, intracellular organic osmolytes in aquatic invertebrates (Gilles, 1987). The total intracellular FAA concentration is known to be directly affected by processes of cell water regulation following osmotic stress and is thus frequently used as an indicator for a species capacity to acclimate to habitat salinity changes Matsushima et al., 1987; Pierce et al., 1992; Jordan and Deaton, 1999; Blank et al., 2004). However, FAA in marine invertebrates are involved in a variety of other environmental and developmental processes and can also be affected by anaerobiotic energy metabolism (Zurburg and De Zwaan, 1981; Powell et al., 1982), food supply (Welborn and Manahan, 1995), parasitism (Paynter et al., 1995), reproduction (Kasshau and McCommas, 1982) and pollutants (Hummel et al., 1996). The interaction of several concomitant acting factors finally leads to a specific qualitative and quantitative FAA composition.

Recent analyses of the FAA concentration in European *M. balthica* and *Mytilus* spp. have shown that the total FAA value is determined by the habitat salinity in populations along the salinity gradient from the oligohaline northern Baltic Sea to the mesohaline Atlantic estuaries whereas salinity conditions above 20 PSU do not cause a corresponding FAA increase (Kube et al., 2006). Alanine, glycine and taurine are identified as the three main FAA in *M. balthica* and *Mytilus* spp. (Kube et al., 2006).

The comparative study of the seasonal variability of FAA in *M. balthica* and *Mytilus* spp. in the present study has revealed that even if salinity is the main factor determining the total FAA concentration in different populations, the seasonal variations of FAA patterns between and within populations could only partly be explained by seasonal salinity fluctuations.

Seasonal FAA changes differ (1) between both studied bivalves and (2) between Baltic, Atlantic and Mediterranean populations.

(1) Differences between *Mytilus* and *Macoma* become apparent in the general composition of the FAA pool: alanine is generally highly concentrated in *M. balthica* whereas taurine shows higher concentrations in *Mytilus* spp. Hence, values of the total FAA pool throughout the year are basically attributed to taurine in *Mytilus* and to a balanced combination of different amino acids in *Macoma*. Differences in the composition of the FAA pool in different species are known for other bivalves as well (Shumway et al., 1977). However, considering the seasonal fluctuations of the total FAA pool in *Mytilus* and *Macoma* from the same sampling sites, comparable patterns were found. Especially in the mesohaline Baltic Sea, the values of FAA concentrations as well as seasonal variations are quite similar in both taxa, suggesting a great physiological plasticity of the processes involved in the amino acid metabolism.

(2) Based on shell morphology and genetic analyses, three different *Mytilus* species are described to occur along the European coast: the Baltic type *M. trossulus*, the Atlantic *Mytilus edulis* and the Mediterranean *M. galloprovincialis* (Varvio et al., 1988; Seed, 1992; Sanjuan et al., 1997; Hummel et al., 2001; Smietanka et al., 2004). Their exact taxonomic status remains controversial, due to the evidence of hybridization and gene introgression in areas were their distribution ranges overlap (Gardner, 1996; Riginos and Cunningham, 2005). Regarding *M. balthica* similar genetic subdivisions as in *Mytilus* have been described. Three different genetic types have been distinguished across Europe: the Baltic type, the Atlantic type and the French type (Luttikhuizen et al., 2003; Vaeinoelae, 2003). At present it is not clear whether the genetically subdivided populations of *Mytilus* and *Macoma* show local adaptations in terms of environmental factors such as salinity and temperature, reflected by variations in the FAA plasticity. Concerning the constitution of the FAA pool, our data show remarkable differences concerning the use of taurine between Baltic and Atlantic populations. However, there is some evidence that taurine accumulation in molluscs depends on dietary supply of taurine precursors such as cysteine and methionine for de novo synthesis or on dietary supply of taurine per se (Bishop et al., 1983; Welborn and Manahan, 1995). Hence, differences in taurine levels between populations might be determined by genetic differences and/or reflect different compositions of diet. Considering the very similar taurine levels in genetically different populations such as *Macoma* from Westerschelde and Bight of Aiguillon and *Mytilus* from Atlantic and Mediterranean populations, the second hypothesis seems to be more likely.

The great seasonal plasticity of FAA patterns in *Macoma* and *Mytilus* along the European coast is suggested to be caused by processes of (1) cell volume regulation, (2) energy metabolism and (3) reproductive cycle.

All three main FAA serve as organic osmolytes and are involved in the processes of isoosmotic cell volume regulation in marine bivalves to balance cell water gain or loss when extracellular osmotic pressure changes (Shumway et al., 1977; Livingstone et al., 1979). We followed FAA changes in *M. balthica* and *Mytilus* spp. from 4 locations exposed to seasonal salinity fluctuations: Mecklenburg Bight, Westerschelde, Bight of Aiguillon and Bidasoa. In *M. balthica*, seasonal patterns of alanine, glycine and taurine run parallel with a peak in winter in Mecklenburg Bight and a decrease of all three FAA in winter in both Atlantic estuaries. The salinity in the south-western Baltic Sea was higher in winter than in summer, due to saline water inflows from the Kattegat into the south-western Baltic during

autumn and winter storm events (Nausch et al., 2004, 2005). In contrast, the salinity in the Atlantic estuaries dropped in winter due to higher precipitation and river discharge (http://www.waterbase.nl,www.ifremer.fr). Thus, the seasonal FAA variations in *M. balthica* from these locations might be explained by the function of FAA for salinity acclimation.

In *Mytilus* spp., processes of salinity acclimatization can only provide an explanation for the variations of glycine and taurine in Mecklenburg Bight. In *M. edulis* from the Atlantic estuaries, no clear salinity dependent FAA variations were obtained. In the Bight of Aiguillon and Bidasoa, only the taurine concentration might be related to habitat salinity fluctuations. The less pronounced FAA–salinity relation in *Mytilus* spp. in comparison to *M. balthica* might be explained by the utilisation of other osmolytes such as betaines to balance seasonal salinity fluctuations in *Mytilus* spp. (De Vooys and Geenevasen, 2002) and/or a higher tolerance to salinity dependent cell volume changes in *Mytilus* spp. The routine absence of substantial cell volume regulation in many tissues of estuarine bivalves such as *Mytilus* spp. may serve to avoid large energetic costs associated with repeated volume regulation in habitats with large and frequent short-term salinity fluctuations (Neufeld and Wright, 1996, 1998).

However, significant seasonal FAA variations in *M. balthica* and *Mytilus* spp. from locations without any intraannual salinity fluctuations (Bothnian Gulf, Askö, Grevelingen, Marseille) show that other factors than salinity changes must affect the FAA values considerably.

In the Bothnian Gulf and Askö, an increase of alanine and glycine was observed in autumn and winter in both bivalve taxa. To explain this phenomenon, the function of FAA in energy yielding metabolic processes might be important. The alanine accumulation in *M. balthica* in winter could result from a switch to a partly anaerobic metabolism during hibernation due to low oxygen availability in ice covered sediments and a general loss of respiratory capacity at low temperatures Poertner, 2002). Those extreme winter climate conditions are especially characteristic for the most northern location in the Bothnian Gulf, where the steepest negative relation between alanine concentration and habitat temperature was observed. Moreover, with increasing alanine concentrations in the northern *M. balthica* populations between October and January, the glycogen values decreased (Fig. 5). Besides nutritional stress and gametogenesis (Hummel et al., 1988), anaerobiosis might be a reason for the observed glycogen decrease and alanine accumulation.

In contrast to the bivalves from the northern Baltic populations, in *Mytilus* spp. from the Atlantic and Mediterranean locations, alanine was positively correlated with habitat temperature. In the Westerschelde and the Bight of Aiguillon this could partly be explained by osmolyte accumulation due to salinity dependent cell volume regulation since both, salinity and temperature were higher in summer than in winter in these estuaries. The positive correlation of temperature and alanine in *M. edulis* from Grevelingen and *M. galloprovincialis* from Marseille however shows that other metabolic processes caused the high alanine values in summer.

Processes of cellular osmotic adjustment and energy metabolism only partly explain the variability of glycine values. The concentration of glycine in Baltic Sea bivalves showed a peak in autumn and winter, in *M. edulis* from Grevelingen and Westerschelde a peak was observed in winter and spring, in *Mytilus* spp. from Aiguillon, Bidasoa and Marseille glycine concentrations were highest in summer. These findings are in accordance with earlier investigations of *M. balthica* in the Bight of Gdansk and *M. edulis* from the Dutch Wadden Sea and Schelde estuary (Zandee et al., 1980; Zurburg et al., 1989; Sokolowski et al., 2003). Kasshau and McCommas (1982) found increasing glycine concentrations in the sea anemone *Bundosoma cavernata* during the gonadal development and gave some indications that glycine might be increase the viability of sperm and facilitate the process of fertilisation. Our investigations do not support this hypothesis for *M. balthica* and *Mytilus* spp. Only for *Mytilus* spp. from the Bight of Aiguillon and Bidasoa a positive correlation of the gonadal index and glycine concentration was observed. For *Mytilus* spp. and *M. balthica* from the Baltic Sea and Grevelingen, glycine decreased with gonadal development. In bivalves from Westerschelde and Marseille no relation of the gonadal index and glycine was found.

In summary, our large scale comparative study of the seasonal variability of FAA in *M. balthica* and *Mytilus* spp. cleary shows that besides salinity, other environmental factors and the physiological state have to be considered to explain variations in FAA patterns between and within populations.

Acknowledgements

We wish to thank A. Gerber, B. Hambach, A. Pronker, C. Sola, M. Markiegi, P. Garcia, R. Lasota, E. Flach, G. Ejdung, P. Magni for their help during the field and laboratory work. We are grateful to H. Hummel and M. Wolowicz as well as to two anonymous referees for their valuable comments to an earlier draft of the manuscript. This study has been carried out in the frame of the project "The impact of biodiversity changes in coastal marine benthic ecosystems" which is funded by the European Commission (Research Directorate General, Environment Programme-Marine Ecosystems) in the Community's Fifth Framework Programme (contract no EVK3-2001-00146).

References

Baginski, R.M., Pierce, S.K., 1977. Time course of intracellular free amino-acid accumulation in tissues of *Modiolus demissus* during high salinity adaptation. Comp. Biochem. Physiol. A 57, 407–412.

Bayne, B.L., Widdows, J., Thompson, R.J., 1976. Physiology II. In: Bayne, B.L. (Ed.), Marine Mussels: Their Ecology and Physiology. Cambridge University Press, Cambridge, pp. 207–260.

Bishop, J.S., Burton, R.S., 1993. Amino-acid synthesis during hyperosmotic stress in *Penaeus-aztecus* postlarvae. Comp. Biochem. Physiol. A 106, 49–56.

Bishop, S.H., Ellis, L.L., Burcham, J.M., 1983. Amino acid metabolism in molluscs. In: Wilbur, K.M. (Ed.), The Mollusca. Metabolism Biochemistry and Molecular Biomechanics, vol. 1. Academic Press, New York, pp. 243–327.

Blank, M., Bastrop, R., Rohner, M., Jurss, K., 2004. Effect of salinity on spatial distribution and cell volume regulation in two sibling species of *Marenzelleria* (Polychaeta: Spionidae). Mar. Ecol., Prog. Ser. 271, 193–205.

Caddy, J.F., 1967. Maturation of gametes and spawning in *Macoma balthica* (L.). Can. J. Zool. 45, 955–965.

Chiou, T.K., Lai, M.M., Shiau, C.Y., 2001. Seasonal variations of chemical constituents in the muscle and viscera of small abalone fed different diets. Fish. Sci. 67, 146–156.

Chipperfield, P.N.J., 1953. Observations on the breeding and settlement of *Mytilus edulis* (L.) in British waters. J. Mar. Biol. Assoc. U.K. 32, 449–476.

Deaton, L.E., Hilbish, T.J., Koehn, R.K., 1985. Hyperosmotic volume regulation in the tissues of the mussel *Mytilus edulis*. Comp. Biochem. Physiol. A 80, 571–574.

De Vooys, C.G.N., 1991. Anaerobic metabolism in sublittoral living *Mytilus galloprovincialis* in the Mediterranean — 4. Role of amino acids in adaptation to low salinities during anaerobiosis and aerobiosis. Comp. Biochem. Physiol. A 100, 423–431.

De Vooys, C.G.N., Geenevasen, J.A.J., 2002. Biosynthesis and role in osmoregulation of glycine-betaine in the Mediterranean mussel *Mytilus galloprovincialis* LMK. Comp. Biochem. Physiol. B 132, 409–414.

de Zwaan, A., 1991. Molluscs. In: Bryant, C. (Ed.), Metazoan Life without Oxygen. Chapman and Hall, pp. 186–217.

Gardner, J.P.A., 1996. The *Mytilus edulis* species complex in southwest England: effects of hybridization and introgression upon interlocus associations and morphometric variation. Mar. Biol. 125, 385–399.

Gäde, G., 1988. Biological role of octopine formation in marine mollusks. Mar. Biol. Lett. 1, 121–135.

Gilles, R., 1987. Volume regulation in cells of euryhaline invertebrates. Curr. Top. Membr. Transp. 30, 205–247.

Goolish, E.M., Burton, R.S., 1989. Energetics of osmoregulation in an intertidal copepod — effects of anoxia and lipid reserves on the pattern of free amino-acid accumulation. Func. Ecol. 3, 81–89.

Helland, S., Triantaphyllidis, G.V., Fyhn, H.J., Evjen, M.S., Lavens, P., Sorgeloos, P., 2000. Modulation of the free amino acid pool and protein content in populations of the brine shrimp *Artemia* spp. Mar. Biol. 137, 1005–1016.

Hülsmann, R., Osinsky, R., Schiedek, D., Schöttler, U., 1991. The importance of proline metabolism for adaptation to low salinity in *Nereis diversicolor* (Polychaeta). Verh. Dtsch. Zool. Ges. 84, 411.

Hummel, H., de Wolf, L., Fortuin, A.W., 1988. The annual cycle of glycogen in estuarine benthic animals. Hydrobiol. Bull. 22, 199–202.

Hummel, H., Bogaards, R., De Wolf, L., Sinke, J., Poortvliet, T., 1994. Evaluation of free amino acids as a biochemical indicator of metal pollution. Mar. Environ. Res. 38, 303–312.

Hummel, H., Amiard-Triquet, C., Bachelet, G., Desprez, M., Marchand, J., Sylvand, B., Amiard, J.C., Rybarczyk, H., Bogaards, R.H., Sinke, J., De Wolf, L., 1996. Free amino acids as a biochemical indicator of stress in the estuarine bivalve *Macoma balthica*. Sci. Total. Environ. 188, 233–241.

Hummel, H., Colucci, F., Bogaards, R.H., Strelkov, P., 2001. Genetic traits in the bivalve *Mytilus* from Europe, with an emphasis on Arctic populations. Pol. Biol. 24, 44–52.

Jordan, P.J., Deaton, L.E., 1999. Osmotic regulation and salinity tolerance in the freshwater snail *Pomacea bridgesi* and the freshwater clam *Lampsilis teres*. Biochem. Physiol. A 122, 199–205.

Kasshau, M.R., McCommas, S.A., 1982. Glycine concentration as a biochemical indicator of sex and maturation in the sea anemone *Bunodosoma cavernata*. Comp. Biochem. Physiol. A 72, 595–597.

Keck, T., Maurer, D., Lind, H., 1975. A comparitive study of the hard clam gonad development cycle. Biol. Bull. 148, 243–258.

Keppler, D., Decker, K., 1984. Glycogen. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis, vol. 6. Verlag Chemie, Weinheim, pp. 11–18.

Koenig, M.L., Powell, E.N., Kasschau, M.R., 1981. The effects of salinity change on the free amino-acid pools of 2 nereid polychaetes, *Neanthes succinea* and *Leonereis culveri*. Comp. Biochem. Physiol. A 70, 631–637.

Kube, S., Gerber, A., Jansen, J.M., Schiedek, D., 2006. Patterns of organic osmolytes in two marine bivalves, *Macoma balthica* and *Mytilus* spp., along their European distribution. Mar. Biol. 149, 1387–1396.

Livingstone, D.R., Widdows, J., Fieth, P., 1979. Aspects of nitrogen metabolism of the common mussel *Mytilus edulis*: adaptation to abrupt and fluctuating changes in salinity. Mar. Biol. 53, 41–55.

Lutikhuizen, P.C., Drent, J., Baker, A.J., 2003. Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. Mol. Ecol. 12, 2215–2229.

Matsushima, O., Hayashi, Y.S., 1992. Metabolism of D-alanine and L-alanine and regulation of intracellular free amino-acid levels during salinity stress in a brackish-water bivalve *Corbicula japonica*. Comp. Biochem. Physiol. A 102, 465–471.

Matsushima, O., Katayama, H., Yamada, K., 1987. The capacity for intracellular osmoregulation mediated by free amino acids in three bivalve molluscs. J. Exp. Mar. Biol. Ecol. 109, 93–99.

Nausch, G., Feistel, R., Lass, H.U., Nagel, K., Siegel, H., 2004. Hydrographisch-chemische Zustandseinschätzung der Ostsee 2003. Meereswiss. Ber. Warnemünde 59, 1–80.

Nausch, G., Feistel, R., Lass, H.U., Nagel, K., Siegel, H., 2005. Hydrographisch-chemische Zustandseinschätzung der Ostsee 2004. Meereswiss. Ber. Warnemünde 62, 1–78.

Neufeld, D.S., Wright, S.H., 1996. Response of cell volume in *Mytilus* gill to acute salinity change. J. Exp. Biol. 199, 473–484.

Neufeld, D.S., Wright, S.H., 1998. Effect of cyclical salinity changes on cell volume and function in *Geukensia demissa* gills. J. Exp. Biol. 201, 1421–1431.

Paynter, K.T., Pierce, S.K., Burreson, E.M., 1995. Levels of intracellular free amino-acids used for salinity tolerance by oysters (*Crassostrea virginica*) are altered by protozoan (*Perkinsus marinus*) parasitism. Mar. Biol. 122, 67–72.

Peng, K.W., Chew, S.F., Ip, Y.K., 1994. Free amino-acids and cell-volume regulation in the sipunculid *Phascolosoma arcuatum*. Physiol. Zool. 67, 580–597.

Pierce, S.K., Rowland-Faux, L.M., O'Brien, S.M., 1992. Different salinity tolerance mechanisms in Atlantic and Chesapeake Bay conspecific oysters — glycine betaine and amino-acid pool variations. Mar. Biol. 113, 107–115.

Poertner, H.O., 2002. Physiological basis of temperature-dependent biogeography: trade offs in muscle-design and performance in polar ectotherms. J. Exp. Biol. 205, 2217–2230.

Powell, E.N., Kasschau, M., Chen, E., Koenig, M., Pecon, J., 1982. Changes in the free amino-acid pool during environmental-stress in the gill tissue of the oyster, *Crassostrea-virginica*. Comp. Biochem. Physiol. A 71, 591–598.

Riginos, C., Cunningham, C.W., 2005. Local adaptation and species segregation in two mussel (*Mytilus edulis* × *Mytilus trossulus*) hybrid zones. Mol. Ecol. 14, 381–400.

Rosa, R., Calado, R., Andrade, A.M., Narciso, L., Nunes, M.L., 2005. Changes in amino acids and lipids during embryogenesis of European lobster, *Homarus gammarus* (Crustacea: Decapoda). Comp. Biochem. Physiol. B 140, 241–249.

Sanjuan, A., Zapata, C., Alvarez, G., 1997. Genetic differentiation in *Mytilus galloprovincialis* Lmk. throughout the world. Ophelia 47, 13–31.

Seed, R., 1992. Systematics evolution and distribution of mussels belonging to the genus *Mytilus*: an overview. Am. Malacol. Bull. 9, 123–137.

Shumway, S.E., Gabbott, P.A., Youngson, A., 1977. Effect of fluctuating salinity on concentrations of free amino-acids and ninhydrin-positive substances in adductor muscles of 8 species of bivalve mollusks. J. Exp. Mar. Biol. Ecol. 29, 131–150.

Smietanka, B., Zbawicka, M., Wolowicz, M., Wenne, R., 2004. Mitochondrial DNA lineages in the European populations of mussels (*Mytilus* spp.). Mar. Biol. 146, 79–92.

Sokolowski, A., Wolowicz, M., Hummel, H., 2003. Free amino acids in the clam *Macoma balthica* L. (Bivalvia, Mollusca) from brackish waters of the southern Baltic Sea. Comp. Biochem. Physiol. A 134, 579–592.

Vaeinoelae, R., 2003. Repeated trans-Arctic invasions in littoral bivalves: molecular zoogeography of the *Macoma balthica* complex. Mar. Biol. 143, 935–946.

Varvio, S.L., Koehn, R.K., Vaeinoelae, R., 1988. Evolutionary genetics of the *Mytilus edulis* complex in the northern Atlantic region. Mar. Biol. 98, 51–60.

Welborn, J.R., Manahan, D.T., 1995. Taurine metabolism in larvae of marine mollusks (Bivalvia, Gastropoda). J. Exp. Biol. 198, 1791–1799.

Wenne, R., 1985. Microgeographic differentiation of the reproductive cycle of *Macoma balthica* (L.) in the Gdansk Bay (South Baltic), and the relationship between this cycle and energy reserve changes. Pol. Arch. Hydrobiol. 32, 47–63.

Yancey, P.H., 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J. Exp. Biol. 208, 2819–2830.

Zandee, D.I., Kluytmans, J.H., Zurburg, W., Pieters, H., 1980. Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. Neth. J. Sea Res. 14, 1–29.

Zurburg, W., De Zwaan, A., 1981. The role of amino-acids in anaerobiosis and osmoregulation in bivalves. J. Exp. Zool. 215, 315–325.

Zurburg, W., Hummel, H., Bogaards, R., De Wolf, L., Ravestein, H., 1989. Free amino acid concentrations in *Mytilus edulis* L. from different locations in the southwestern part of the Netherlands: their possible significance as a biochemical stress indicator. Comp. Biochem. Physiol. A 93, 413–417.