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# Heterothermal acclimation: An experimental paradigm for studying the control of thermal acclimation in crabs

MIRELA CUCULESCU, TIMOTHY PEARSON, DAVID HYDE, AND KEN BOWLER*

Department of Biological Sciences, The University of Durham, Durham City DH1 3LE, United Kingdom

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**ABSTRACT** **A method for the study of the control of the attainment of thermal acclimation has been applied to the crabs, *Cancer pagurus* and *Carcinus maenas*. Crabs were heterothermally acclimated by using an anterior–posterior partition between two compartments, one at 8°C and the other at 22°C. One compartment held a three-quarter section of the crab including the central nervous system (CNS), eye stalks, and ipsilateral legs; the other held a quarter section including the contralateral legs. Criteria used to assess the acclimation responses were comparisons of muscle plasma membrane fatty acid composition and "fluidity." In both species, the major fatty acids of phosphatidylcholine were 16:0, 18:1, 20:5, and 22:6, whereas phosphatidylethanolamine contained significantly less 16:0 but more 18:0; these fatty acids comprised 80% of the total. Differences in fatty acid composition were demonstrated between fractions obtained from the ipsilateral and contralateral legs from the same heterothermally acclimated individual. In all acclimation states (except 22CNS, phosphatidylcholine fraction), membrane lipid saturation was significantly increased with acclimation at 22° as compared with 8°C. Membrane fluidity was determined by using 1,3-diphenyl-1,3,5 hexatriene (DPH) fluorescence polarization. In both species, membranes from legs held at 8° were more fluid than from legs held at 22°C irrespective of the acclimation temperature of the CNS. Heterothermal acclimation demonstrated that leg muscle membrane composition and fluidity respond primarily to local temperature and were not predominately under central direction. The responses between 8°C- and 22°C-acclimated legs were more pronounced when the CNS was cold-acclimated, so a central influence cannot be excluded.**

Poikilotherms generally inhabit environments subject to daily and seasonal temperature change, and thus, their body temperatures will vary with time. Many poikilotherms have been shown to possess a suite of behavioral, structural, physiological, and biochemical responses that enable them to compensate for the changes in functioning that follow from such fluctuations in ambient temperature (1).

One clear outcome of a change in body temperature of a poikilotherm is the perturbation of cell membrane physical state. A rise in temperature will increase the rate and extent of phospholipid acyl chain motion, whereas a decrease in temperature will increase the ordering of membrane phospholipids. Changes in phase may also occur, altering the distribution of microdomains. Such temperature-induced changes in the organization of the lipid bilayer may have consequences for function.

Poikilotherms have the ability to remodel membrane lipids in response to a change in prevailing temperature so that membrane physical properties remain appropriate. This remodeling has been shown to involve phospholipid head group composition (2), acyl chain unsaturation (3, 4), molecular species composition (5–7), and cholesterol content (8), a field recently reviewed (9). It is generally considered that this remodeling serves to maintain a relatively constant physical state for the membrane lipids (homeoviscous adaptation; refs. 10 and 11) over the range of temperatures experienced, implying a role for homeoviscous adaptation that requires membrane function to be influenced by membrane lipid order (12). This widely reported phenomenon occurs in a variety of animal groups (13), as well as in plants (14) and bacteria (10). Indeed, our studies have demonstrated that changes in membrane unsaturation and cholesterol/phospholipid ratios, as well as in membrane physical state, occur in the brachyuran crabs *Carcinus maenas* and *Cancer pagurus* and correlate with thermal acclimation (15).

Of the divers responses that have been studied in thermal acclimation, these graded changes in membrane lipid composition and physical state are probably the best documented (3). What is not resolved is how this process of thermal acclimation in poikilotherms is controlled. A significant role has been demonstrated for the influence of the nervous and endocrine systems (16–19); however, other reports demonstrate that isolated cells in culture are capable of thermal acclimation (20, 21). To assess the relative contributions made by hormonal/central nervous system (CNS) influences vs. local independent cellular responses to the attainment of acclimation in an intact organism, we have developed an approach that uses a heterothermal model that allows, during acclimation, lateral thermal partitioning of a crab so that the CNS and ipsilateral legs are at one temperature while the contralateral legs are held at another temperature. This separates central from local thermal influences in the attainment of acclimation. The protocol has been used successfully to monitor acclimation-driven responses in neurophysiological parameters, and the present work reports on the responses in walking leg muscle membrane lipid composition and physical state.

## MATERIALS AND METHODS

**Animals.** Crabs (*C. maenas* and *C. pagurus*) were caught locally in the North Sea, and animals of 10-cm carapace width were held in filtered sea water at 8°C until used.

**Heterothermal Acclimation.** A rubber diaphragm was positioned anterior–posterior to separate laterally a three-quarter section of the crab that included the CNS and both eye stalks from the contralateral quarter section. The rubber diaphragm formed a partition between two, temperature-controlled seawater compartments of the heterothermal apparatus; one compartment was held at 8°C while the contralateral compartment was held at 22°C; both the central and outer compartments of the water bath were aerated.

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Abbreviations: CNS, central nervous system; PE, phosphatidylethanolamine; PC, phosphatidylcholine; FA, fatty acid; S/U, saturated FA/unsaturated FA.

*To whom reprint requests should be addressed. e-mail: Kenneth.Bowler@Durham.ac.uk.

Table 1. FA composition of the PC and PE classes of phospholipid extracted from plasma membranes of *C. pagurus* walking legs of crabs heterothermally acclimated with their CNS at 8°C

| | PC | | PE | |
|---|---|---|---|---|
| FA | 8° | 22° | 8° | 22° |
| 14:1 | 0.18 ± 0.04 | 0.13 ± 0.05* | 0.10 ± 0.05 | 0.23 ± 0.16 |
| 16:0 | 14.45 ± 0.88 | 15.95 ± 0.64** | 6.66 ± 0.4 | 8.36 ± 0.86* |
| 16:1 | 6.92 ± 0.31 | 5.95 ± 0.46* | 2.62 ± 0.19 | 3.17 ± 0.33 |
| 17:1 | 1.97 ± 0.18 | 2.04 ± 0.33 | 2.61 ± 0.43 | 2.69 ± 0.61 |
| 18:0 | 1.39 ± 0.11 | 2.38 ± 0.25** | 6.01 ± 0.53 | 7.9 ± 0.55** |
| 18:1 | 24.03 ± 0.76 | 25.68 ± 0.41 | 21.36 ± 1 | 19.62 ± 1.69 |
| 20:4 | 3.57 ± 0.4 | 3.62 ± 0.51 | 4.65 ± 0.4 | 4.83 ± 0.59 |
| 20:5 | 24.65 ± 0.76 | 20.91 ± 0.71* | 29.7 ± 0.59 | 25.43 ± 1.18** |
| 24:1 | 0.84 ± 0.07 | 1.02 ± 0.21 | 0.52 ± 0.09 | 0.78 ± 0.4 |
| 22:6 | 12.81 ± 0.32 | 12.48 ± 0.43 | 18.17 ± 0.75 | 17.07 ± 1.02 |
| Saturated FA | 19.69 ± 1.13 | 22.16 ± 0.72* | 15.67 ± 0.61 | 19.4 ± 0.72** |
| MUFA | 35.44 ± 0.56 | 36.56 ± 0.39 | 28.2 ± 0.7 | 28.84 ± 1.5 |
| PUFA | 44.09 ± 0.7 | 40.07 ± 0.85** | 54.54 ± 0.77 | 51.7 ± 1.73 |
| S/U | 0.246 ± 0.02 | 0.29 ± 0.001* | 0.189 ± 0.01 | 0.242 ± 0.01** |

Values are given as mean ± SEM. MUFA, monounsaturated FA; PUFA, polyunsaturated FA. *, $P < 0.05$; **, $P < 0.01$. $n = 6$.

The central compartment was held at either 22°C or 8°C acclimation temperature, and for each protocol, the contralateral legs were held at either 8°C or 22°C, thus permitting the following combinations of leg and CNS acclimation temperatures: 8CNS/8Leg and 8CNS/22Leg or 22CNS/22Leg and 22CNS/8Leg. The crabs were held in the heterothermal apparatus for 3 weeks to allow completion of acclimation.

**Plasma Membrane Isolation.** Plasma membranes were prepared from 4–7 g of muscle (wet weight) from walking legs of crabs as described (22).

**Membrane Fluidity Measurements.** Thawed plasma membrane suspensions in 10 mM phosphate buffer (pH 7.6) were sonicated on ice and mixed with 2 μl of a solution of 2 mM 1,3-diphenyl-1,3,5 hexatriene (DPH) in tetrahydrofuran and incubated for 10 min at room temperature. Steady-state fluorescence polarization was measured at 5, 15, and 20°C. The equipment used and the calculations are as described (23).

**Lipid Extraction and Analysis.** Total lipids were extracted from freshly prepared plasma membranes by using the method of Bligh and Dyer (24). Individual phospholipid classes were separated by using two-dimensional TLC, and total phospholipid was determined with a mixture of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (2:1 wt/wt) as standard (25). Methyl esters were prepared (26) and separated by using a Shimadzu GC-9A series gas chromatograph (2 m column, 2 mm i.d., packed with cyanosilicone stationary phase, 105 Alltech CS-5, on a Chromabsorb WAW 100–120 mesh support). Peaks were identified by comparison with authentic standards and their relative retention times.

**Statistical Analysis.** The significant difference of mean values obtained from the ipsi- and contralateral leg muscle of separate heterothermal experiments were compared by using paired Student's *t* test.

## RESULTS

Tables 1–4 show the fatty acid (FA) composition of PC and PE phospholipids from walking leg muscle plasma membranes of heterothermally acclimated *C. pagurus* and *C. maenas.* Only the major FA and those minor FA where significant differences were found are included in the Tables. The data were consistent between species and experimental conditions in that the dominant FA of PC were 16:0, 18:1, 20:5, and 22:6, which composed some 80% of the total; however, PE had significantly less 16:0 but more 18:0 than PC.

Table 1 shows the data for *C. pagurus* with CNS acclimated at 8°C. The FA composition of the 22°C- and 8°C-acclimated legs differ in both the PC and PE fractions. There were

Table 2. Fatty acid composition of the PC and PE classes of phospholipid extracted from plasma membranes of *C. pagurus* walking legs of crabs heterothermally acclimated with their CNS at 22°C

| | PC | | PE | |
|---|---|---|---|---|
| FA | 8° | 22° | 8° | 22° |
| 14:1 | 0.16 ± 0.09 | 0.17 ± 0.04 | 0.04 ± 0.04 | 0.14 ± 0.08 |
| 16:0 | 15.1 ± 1.44 | 15.81 ± 0.62 | 8.12 ± 0.48 | 8.23 ± 0.8 |
| 16:1 | 6.94 ± 0.62 | 5.45 ± 0.41 | 3.22 ± 0.22 | 2.69 ± 0.46 |
| 17:1 | 2.02 ± 0.2 | 1.8 ± 0.19* | 2.34 ± 0.43 | 2.65 ± 0.48 |
| 18:0 | 1.97 ± 0.21 | 2.14 ± 0.43 | 6.41 ± 1.27 | 9.15 ± 1.01* |
| 18:1 | 23.04 ± 0.85 | 26.68 ± 1.08** | 22.0 ± 1.82 | 18.78 ± 2.29* |
| 20:4 | 3.63 ± 0.33 | 3.51 ± 0.36 | 4.5 ± 0.21 | 5.03 ± 0.34 |
| 20:5 | 24.93 ± 2.14 | 20.33 ± 0.22 | 26.52 ± 1 | 25.51 ± 0.31 |
| 22:6 | 13.7 ± 0.4 | 13.38 ± 0.13 | 17.85 ± 0.99 | 18.87 ± 0.76 |
| SFA | 20.01 ± 2.01 | 21.51 ± 1.17 | 16.75 ± 1.35 | 20.07 ± 1.04** |
| MUFA | 34.4 ± 1.34 | 36.25 ± 0.8 | 29.99 ± 1.28 | 26.11 ± 1.11** |
| PUFA | 44.68 ± 2.4 | 39.92 ± 0.4 | 51.31 ± 1.15 | 51.91 ± 0.76 |
| S/U | 0.255 ± 0.05 | 0.285 ± 0.02 | 0.208 ± 0.02 | 0.255 ± 0.02* |

Values are given as mean ± SEM. Abbreviations are as in Table 1. *, $P < 0.05$; **, $P < 0.01$. $n = 6$.

Table 3. Fatty acid composition of the PC and PE classes of phospholipid extracted from plasma membranes of *C. maenas* walking legs of crabs heterothermally acclimated with their CNS at 8°C

| | PC | | PE | |
|---|---|---|---|---|
| FA | 8° | 22° | 8° | 22° |
| 16:0 | 16.2 ± 0.63 | 17.53 ± 0.63** | 7.65 ± 0.23 | 9.03 ± 0.59** |
| 16:1 | 9.37 ± 0.32 | 6.68 ± 0.22*** | 4.04 ± 0.23 | 3.34 ± 0.32 |
| 17:1 | 1.77 ± 0.19 | 1.38 ± 0.23** | 1.6 ± 0.07 | 1.21 ± 0.16* |
| 18:0 | 1.46 ± 0.08 | 2.29 ± 0.16** | 5.18 ± 0.11 | 5.37 ± 0.22 |
| 18:1 | 26.23 ± 0.37 | 24.24 ± 0.77** | 19.54 ± 0.43 | 17.39 ± 0.64* |
| 20:4 | 2.87 ± 0.76 | 2.87 ± 0.68 | 4.68 ± 1.05 | 3.9 ± 0.7 |
| 20:5 | 21.62 ± 0.91 | 21.41 ± 0.98 | 28.23 ± 0.85 | 26.35 ± 1.43 |
| 22:6 | 10.0 ± 0.75 | 12.1 ± 1.24* | 19.09 ± 1.15 | 20.57 ± 2.1 |
| SFA | 21.22 ± 1.2 | 23.7 ± 1.16*** | 16.12 ± 0.56 | 18.22 ± 0.9* |
| MUFA | 39.46 ± 2.69 | 34.9 ± 0.57*** | 27.14 ± 0.82 | 25.22 ± 0.55 |
| PUFA | 36.91 ± 0.95 | 39.09 ± 1.07* | 54.67 ± 1.18 | 54.89 ± 0.7 |
| S/U | 0.271 ± 0.02 | 0.319 ± 0.02** | 0.192 ± 0.01 | 0.230 ± 0.01* |

Values are given as mean ± SEM. Abbreviations are as in Table 1.*$P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. $n = 6$.

significant differences in a number of minor FA, but the major difference is that the 8°C-acclimated leg membranes have more 20:5. Significant increases also were found in 16:0 and 18:0 in both PE and PC fractions from legs acclimated at 22°C. The total FA composition of both the PC and PE fractions from muscles of 8°C-acclimated legs contain significantly less saturated FA and more polyunsaturated FA than those of 22°C-acclimated legs, making the saturated FA/unsaturated FA (S/U) index significantly lower in the former. Table 2 shows data for *C. pagurus* with CNS acclimated at 22°C. In the PC fractions of the main FAs, 18:1 was significantly greater in the 22°C- as compared with the 8°C-acclimated legs. Of the minor FAs, 17:1 was significantly increased in the 8°C legs. In the PE fractions, there was a significant increase in 18:0 and a reduction in 18:1 in the 22°C-acclimated leg. In comparing the total FA composition, there were no significant differences in any of the factors in the PC fraction, but the PE fraction from the 22°C-acclimated leg contained significantly more saturated FA and less monounsaturated FA than from the 8°C-acclimated leg, making the S/U ratio significantly lower in the latter. Table 3 shows that data from *C. maenas* with CNS acclimated at 8°C. The major FA of PC to show significant differences between the 22°C- and 8°C-acclimated legs were 16:0, 16:1, and 18:1, with the level of unsaturated FA being greater in the membranes from the 8°C-acclimated legs; however, the level of 18:0 and 22:6 was greater in the 22°C legs. A small significant increase was also obtained in 17:1 in 8°C legs. The PE fraction showed fewer differences, containing higher 16:0 and lower 17:1 and 18:1 levels in the 22°C-acclimated leg muscle membranes. In comparing the total FA composition of the PC fraction, that from the 22°C acclimated legs contained significantly more saturated FA and polyunsaturated fatty acid, but less monounsaturated FA, and had a significantly higher S/U index. The total FA composition of the PE fraction from the 22°C-acclimated legs was also more saturated and had a significantly larger S/U index than that of the 8°C-acclimated leg. Table 4 shows the data from *C. maenas* with CNS acclimated at 22°C. For PC, no differences were found between the major FA from the 22°C- and 8°C-acclimated legs; however, 14:0 was lower and 18:0 was higher in the warm leg preparations. More change was found for the PE fraction. Of the major FA, 16:0 was significantly higher in the 22°C acclimated legs and 20:5 was significantly lower than from the 8°C-acclimated legs. Of the minor FA, increases also were found in 18:3, in 20.3, and in 22:0 in the warm-acclimated leg as compared with the 8°-acclimated leg. In neither of the

Table 4. FA composition of the PC and PE classes of phospholipid extracted from plasma membranes of *C. maenas* walking legs of crabs heterothermally acclimated with their CNS at 22°C

| | PC | | PE | |
|---|---|---|---|---|
| FA | 8° | 22° | 8° | 22° |
| 14:0 | 0.82 ± 0.16 | 0.56 ± 0.2** | 0.2 ± 0.11 | 0.19 ± 0.14 |
| 16:0 | 16.34 ± 0.4 | 15.88 ± 0.59 | 7.58 ± 0.39 | 9.81 ± 0.68* |
| 16:1 | 8.05 ± 0.81 | 7.61 ± 0.4 | 3.91 ± 0.28 | 4.07 ± 0.36 |
| 17:1 | 2.07 ± 0.57 | 1.65 ± 0.42 | 1.57 ± 0.47 | 1.31 ± 0.47 |
| 18:0 | 1.64 ± 0.2 | 2.53 ± 0.22*** | 4.87 ± 0.15 | 5.59 ± 0.52 |
| 18:1 | 24.35 ± 1.36 | 25.58 ± 1.27 | 18.74 ± 0.66 | 17.33 ± 0.76 |
| 18:3 | 1 ± 0.25 | 1.07 ± 0.24 | 0.92 ± 0.22 | 1.87 ± 0.4** |
| 20:3 | 0.2 ± 0.05 | 0.68 ± 0.34 | 0.24 ± 0.13 | 0.42 ± 0.15* |
| 20:4 | 3.47 ± 0.31 | 3.63 ± 0.35 | 4.63 ± 0.45 | 4.81 ± 0.26 |
| 20:5 | 23.84 ± 1.49 | 22.06 ± 1.02 | 29.18 ± 0.32 | 26.99 ± 0.53* |
| 22:0 | 0.36 ± 0.07 | 0.48 ± 0.22 | 0.51 ± 0.31 | 0.99 ± 0.21* |
| 22:6 | 8.19 ± 1.08 | 8.17 ± 0.5 | 16.69 ± 1.83 | 15.82 ± 2.49 |
| SFA | 22.13 ± 0.6 | 22.61 ± 0.82 | 16.66 ± 0.97 | 19.64 ± 2.02 |
| MUFA | 37.16 ± 1.18 | 37.81 ± 0.92 | 27.5 ± 1.13 | 25.77 ± 0.54 |
| PUFA | 38.31 ± 1.57 | 37.53 ± 0.82 | 53.82 ± 1.72 | 52.33 ± 2.09 |
| S/U | 0.292 ± 0.01 | 0.3 ± 0.01 | 0.205 ± 0.014 | 0.26 ± 0.03* |

Values are given as mean ± SEM. Abbreviations are as in Table 1. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. $n = 5$.

Table 5. Temperature dependence of fluorescence polarization of DPH probes in plasma membranes from the walking legs of heterothermally acclimatized *C. pagurus* and *C. maenas*

| Temperature, °C | *C. pagurus* | | | | *C. maenas* | | | |
|---|---|---|---|---|---|---|---|---|
| | 8CNS | | 22CNS | | 8CNS | | 22CNS | |
| | 8Leg | 22Leg | 8Leg | 22Leg | 8Leg | 22Leg | 8Leg | 22Leg |
| 8 | 0.285 ± 0.005 | 0.295 ± 0.007** | 0.286 ± 0.004 | 0.296 ± 0.006 | 0.291 ± 0.008 | 0.305 ± 0.009* | 0.303 ± 0.007 | 0.323 ± 0.008** |
| 15 | 0.256 ± 0.006 | 0.265 ± 0.007* | 0.260 ± 0.005 | 0.268 ± 0.006** | 0.262 ± 0.007 | 0.298 ± 0.009** | 0.278 ± 0.008 | 0.298 ± 0.009** |
| 22 | 0.233 ± 0.007 | 0.242 ± 0.007** | 0.238 ± 0.005 | 0.245 ± 0.007 | 0.239 ± 0.008 | 0.256 ± 0.009** | 0.254 ± 0.009 | 0.275 ± 0.008** |

*, $P < 0.05$; **, $P < 0.001$.

PL fractions were there any significant differences in the total FA comparisons; however, the S/U index of the PE fraction was significantly lower in the 8°- as compared with the 22°C-acclimated legs.

Table 5 shows the DPH fluorescence polarization data for plasma membranes from the leg muscles of heterothermally acclimated *C. pagurus* and *C. maenas* as a function of measuring temperature. In all cases, polarization values were reduced with an increase in temperature. In both species, the membranes from 8°C-acclimated legs were more fluid than from 22°C-acclimated legs irrespective of the acclimation temperature of the CNS. The efficacy of the acclimation response was not affected by the temperature of the CNS. In *C. pagurus*, the efficacy was 17 ± 3.23 and 17.52 ± 1.22 for the 8CNS- and 22CNS-maintained crabs, respectively. For *C. maenas*, the response was greater, 32.07 ± 4.34 and 42.15 ± 10.07 for the 8CNS- and 22CNS-acclimated crabs, respectively.

## DISCUSSION

Evidence from studies on many species, particularly of marine and freshwater fish, confirm that a consistent response to a lowering of environmental temperature was to modify the lipid composition of cellular membranes (9). The common response was to increase the incorporation of unsaturated FA into phospholipids, rendering the membrane more fluid, which is considered to be a compensation for the direct effect of the lower temperature on membrane structure (11). This compensatory response has been shown to be graded, that is, the extent of the remodeling of the lipids is related to the magnitude of the temperature shift (27). Such changes in membrane lipids are therefore considered to be a reliable index of an acclimation response to temperature.

Remodeling of membrane lipids also is found in marine and freshwater crustaceans. A consistent feature is that the S/U ratio decreases with cold acclimation, resulting from a fall in the proportion of saturated FA and an increase in polyunsaturated FA (28–32). It is pertinent to compare the present data with those previously obtained from the same preparations from crabs acclimated at either 8 or 22°C. For both species, the data presented in Tables 1–4 were similar to those reported earlier for crabs (acclimated at 8°C or 22°C) in that 16:0, 18:0; 18:1; 20:4, 20:5, and 22:6 were the most common FA and that they were present in similar relative proportions (15). A seasonal effect was previously observed on the FA composition of both species, most noticeable in preparations from the crabs caught in spring that had more saturated and less polyunsaturated FA than from crabs caught in autumn. The variation in PL FA composition between individuals was also appreciable, and in consequence, thermal acclimation was found to have no consistent effect on the FA composition of either PC or PE from both species, and no effect was observed on the S/U ratio.

More definitive effects were seen in the present study in FA composition of both PC and PE in response to acclimation temperature. In general, in heterothermally acclimated *C. pagurus*, the 22°C-acclimated leg preparation contained more saturated FA and less unsaturated FA compared with 8°C-acclimated legs so that the S/U ratio was significantly higher. This effect was more pronounced in the crabs that had been acclimated with a cold CNS. A similar effect was observed with preparations from heterothermally acclimated *C. maenas*; however, in preparations from crabs with 22CNS, few significant differences were observed between the cold- and warm-acclimated legs. The major point of emphasis, however, is that some lipid compositional changes could be demonstrated between the warm- and cold-acclimated legs irrespective of the CNS acclimation temperature. The ability to demonstrate these differences in heterothermally acclimated as compared with 8°C and 22°C homothermally acclimated crabs (15) is because the variation in lipid composition between individuals is less important when comparing the ipsi- and contralateral legs from the same individual.

Table 5 also shows clear significant differences in fluorescence polarization of membranes prepared from the crabs heterothermally acclimated at 8°C and 22°C. Similar differences were obtained with membranes obtained from crabs homothermally acclimated at 8°C or 22°C (15). Membranes obtained from legs acclimated at 8°C were consistently less ordered than those from the contralateral legs acclimated at 22°C. Whereas the temperature of the CNS had little influence on this acclimation effect in *C. maenas*, the acclimation effect was more pronounced in crabs heterothermally acclimated with 8CNS than 22CNS in *C. pagurus*.

In conclusion, in crabs, the qualitative responses made at the local level in membrane lipid composition and physical state to a change in ambient temperature are not predominantly under central direction. However, it is not possible to exclude totally a central influence, because in both species the extent of differences between the 8°C and 22°C legs depended on the temperature of the CNS, the differences being more pronounced when the CNS was cold-acclimated.

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