VITAMIN A AND CAROTENOIDs IN CERTAIN INVERTEBRATES

II. STUDIES OF SEASONAL VARIATIONS IN SOME MARINE CRUSTACEA

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(Text-figs. 1–4)

INTRODUCTION

We have previously discussed the richness in vitamin A of the euphausiids, *Meganyctiphanes norvegica* (M. Sars) and *Thysanoessa raschii* (M. Sars), (Fisher, Kon & Thompson, 1952, 1953). We found that astaxanthin was the principal carotenoid pigment and that β-carotene was present in minute amounts, or not at all, in free-swimming animals of these two species, in *Meganyctiphanes norvegica* taken from the stomachs of arctic baleen whales (Kon & Thompson, 1949a) and in *Euphausia superba* Dana from the stomachs of antarctic baleen whales (Kon & Thompson, 1949b).

Our findings contradicted those of Wagner (1939), who had claimed to have found high concentrations of β-carotene in euphausiids from the stomachs of arctic whales, but made no mention of the typical crustacean pigment, astaxanthin.

Moore (1950), in discussing our work, stated that ‘in view of Goodwin’s observations on the variations in the relative concentrations of astaxanthin and β-carotene in locusts it might be unwise to discredit Wagner’s claim to have isolated β-carotene from krill without fully exploring the effects of age and season’. During his study of locusts, Goodwin (1950) found that, in developing eggs, β-carotene was present initially but was gradually replaced from the 6th or 7th day of incubation by astaxanthin. As the hoppers approached maturity, however, the astaxanthin content remained steady whereas β-carotene increased, especially so in the adult phases.

The work described in this paper was designed among others to study whether similar variations in carotenoids occurred in some euphausiid and decapod Crustacea, and whether in this way Wagner’s findings could be reconciled with ours.

MATERIAL AND METHODS OF COLLECTION

A series of collections of *Meganyctiphanes norvegica* and *Thysanoessa raschii* was taken from Loch Fyne at about monthly intervals for 18 months, and the
analytical results have now provided information about possible seasonal and developmental variations in the concentrations of vitamin A and carotenoids. Similar studies were made on *Nephrops norvegicus* Leach and *Crangon allmani* Kinahan taken during the same period from the Clyde Sea area, on *C. vulgaris* L. from Plymouth and Conway and on the eyes of *Homarus vulgaris* M.-Edwards.

The euphausiids, *Meganyctiphanes norvegica* and *Thysanoessa raschii*, were taken from lower Loch Fyne with a 1 m stramin net at depths of 80–90 fathoms (146–165 m) at approximate intervals of 1 month from February 1951 until July 1952. Before the animals were killed each monthly haul was separated into size-groups by length, measured from the tip of the rostrum to the base of the telson, at 2 mm intervals. Intermediate lengths were grouped with the next smaller size.

Collections of *Crangon allmani* and *Nephrops norvegicus* were also made during these visits to Loch Fyne, the former caught with the Agassiz trawl and the latter with the otter trawl. Groups of the brown shrimp, *Crangon vulgaris*, were sent to us at frequent intervals, at first from the Plymouth Laboratory and later from the Fisheries Experiment Station at Conway, during the period from August 1951 to February 1953. One group was collected at Burnham-on-Crouch in March 1952.

All these specimens were preserved as soon as possible after catching by boiling in sea-water, as described by Fisher *et al.* (1952). The euphausiids and *Crangon spp.* were separated into eyes and bodies before analysis and the specimens of *Nephrops* were dissected into several parts. We were also supplied with eyes of *Homarus vulgaris* taken from lobsters boiled for catering.

**Analytical Methods**

Fat was extracted, and vitamin A and carotenoids separated from it by saponification and chromatography on alumina columns, as outlined by Fisher *et al.* (1952). Total carotenoids were measured with the photoelectric spectrophotometer of Thompson (1949), and vitamin A was determined by measurement with this instrument of the intensity of the blue colour produced in the Carr-Price reaction.

Vitamin A ester and alcohol were separated by chromatography before saponification (see Fisher *et al.*, 1952) in the analyses of groups of *Thysanoessa raschii* collected on 5–6 September 1951, and of both this species and *Meganyctiphanes norvegica* collected on 17–18 October 1951, and in subsequent months. Similar treatment was given to some groups of the decapod Crustacea.

We have also analysed groups of eyes of *M. norvegica* for retinene by a method used successfully to separate this compound from cephalopod eyes, to be fully described in a later paper. No retinene was detected in the eyes of *Meganyctiphanes*. 
RESULTS

Euphausiacea

The graphs in Figs. 1 and 2 show the vitamin A and astaxanthin concentrations in relation to the length of the specimens in each size-group of M. norvegica (Fig. 1) and Thysanoessa raschii (Fig. 2). The average weight per specimen of each size-group is also plotted. The detailed results of all the analyses on which these graphs are based have been recorded by Fisher (1953).

Vitamin A was found predominantly in the eyes of both species, varying from 66 to 100% of the total quantity in Meganyctiphanes norvegica and from 65 to 98% in Thysanoessa raschii, and amounting usually to over 90%. Astaxanthin or its esters were the only carotenoids in any of the size groups of either species. In Meganyctiphanes the eyes contained from 18 to 77% of this pigment, the average proportion being about 50% of the total in the whole animal; in Thysanoessa, from 33 to 94% of the total astaxanthin was in the eyes, the average value being about 65%.

As mentioned already, most of the specimens were subjected to the full analytical treatment of Fisher et al. (1952) and the relative proportions of vitamin A alcohol and ester were determined. In T. raschii collected in September the ratio of vitamin A ester to alcohol varied between 6:1 and 28:1 in the eyes of different size-groups and between 1:1 and 2:1 in the bodies; in October the eyes had from 6:1 to 11:1 and the bodies 1:1 to 3:1. In the eyes of Meganyctiphanes norvegica in October, the ratio varied between 2:1 and 16:1, being about 6:1 in most groups, whereas in the bodies it was 1:1 in all groups except one in which it was 3:1. These considerably higher ratios of vitamin A ester to vitamin A alcohol in the eyes than in the bodies observed during September and October were also found in all the remaining groups analysed until the investigation ended in July 1952.

The size-groups studied included the later furcilia stages through to the largest adult forms. In none of the groups of either species was β-carotene ever found in more than minute traces. There was certainly never enough β-carotene to measure, and the measurement would have been possible even if only 1 μg of β-carotene had been present in an extract from several thousand euphausiids. It is unlikely, therefore, that β-carotene is ever present in M. norvegica and Thysanoessa raschii in quantities that would justify Wagner’s (1939) claim.

In many of the groups of Meganyctiphanes norvegica collected from January to July 1952 and in those of Thysanoessa raschii collected from March to July 1952 the sexes were analysed separately, but no consistent differences between them in their contents and concentrations of vitamin A and astaxanthin were found and indeed, on many occasions, the results for the two sexes were remarkably close. We have obtained similar results for Meganyctiphanes norvegica collected at Monaco (Fisher et al., 1953).
Fig. 1. Relationship between body-weight and concentrations of vitamin A and astaxanthin, and length, in *Meganyciphanes norvegica*. V, vitamin A; A, astaxanthin; ——, vitamin A; - - - - - , astaxanthin; . . . . , weight.
Fig. 2. Relationship between body-weight and concentrations of vitamin A and astaxanthin, and length, in *Thysanoessa raschii*. V, vitamin A; A, astaxanthin; W, weight; ---, vitamin A; ----, astaxanthin; ...., weight.
Many of the graphs in Fig. 1 show similarities in the fluctuations of the vitamin A and astaxanthin curves for *M. norvegica*. The concentrations reached their lowest levels in most of the monthly hauls in the size-groups between 20 and 30 mm. In the larvae concentrations were often very high, and in specimens over 30 mm long they tended to rise again, often quite steeply in some of the larger sizes. Unfortunately, after July 1951, only a very few specimens of *M. norvegica* longer than 30 mm were caught and no more good hauls of very large animals were obtained, such as were taken (Fisher et al., 1952) during the autumn and winter of 1950–1, including February 1951, for which results appear in Fig. 1.

Although the vitamin A and astaxanthin curves showed parallel fluctuations, the relative values for astaxanthin became steadily higher during the period February–May 1951, whereas those for vitamin A remained at about the same level. From July until September 1951 the astaxanthin concentrations decreased, but those of vitamin A continued at their previous values. A second, but smaller rise in astaxanthin reserves was detectable during the next few months, reaching its peak in December 1951 or January 1952. The reserves declined slightly during the following period until the study ended in July 1952, and the increase in concentration observed during the spring of the previous year was not repeated. Vitamin A levels tended to be rather higher in June and July 1952 than at any time before.

The curves for concentrations of vitamin A and astaxanthin in *Thysanoessa raschii* (Fig. 2) fluctuated in much the same way as for *Meganyctiphanes norvegica*. Both substances tended to reach their lowest levels in animals between 15 and 19 mm long. The concentrations were highest in the larvae and in specimens over 19 mm in length. From February to July 1951 the concentrations of vitamin A and astaxanthin became lower and then remained at a fairly constant level until October, when they began to rise again to a maximum in January or February 1952. The decline observed in the previous spring was repeated in 1952, astaxanthin reaching its lowest level in June and increasing again in July, and vitamin A being at a minimum in July.

The graphs in Figs. 1 and 2 provide a useful picture based on analyses of the monthly collections, but, since the length-weight relationship changes seasonally as the animals feed and grow or fast and live on their reserves when food is scarce in the winter, seasonal variations can probably be studied better by following through the year one particular size-group. Length, rather than weight, is a better criterion of size because the girth may vary but the animals never become shorter and eventually, if not continuously, increase in length. In neither *M. norvegica* nor *Thysanoessa raschii* is the breeding period so restricted that all animals are simultaneously of the same size. Genetical and environmental variations would soon produce size differences even if this were so. *Meganyctiphanes norvegica* (Poulsen, 1926) and *Thysanoessa raschii* (Einarsson, 1945) usually breed from about March to June. The breeding
periods appear to reach a peak, however, since, in our hauls of both species one size was almost always present in larger numbers than others. The 1 m stramin net was, therefore, regarded as a random sampler of the available population since it is likely that only very small larvae would escape from it. All the specimens in each haul were sorted into size-groups, as far as practicable, except when vast numbers of tiny larvae were taken. This method of selection did not take into account vertical or horizontal movements of populations within, and to and from, Loch Fyne, but euphausiids are caught there regularly and abundantly only in the deepest parts, and it is likely that a population would not move out entirely from an obviously favourable environment. With these reservations, we have considered the values for the size-group with the largest number of specimens in each monthly haul and regarded the series as representing the development of a homogeneous population. The fact that the length of the animals in the maximum-number groups either increased steadily or remained stationary for certain periods, but never regressed, during the year speaks for the soundness of this supposition. From the values so selected graphs were drawn showing the variations in vitamin A and astaxanthin content and concentration in *Meganyctiphanes norvegica* (Fig. 3) and *Thysanoessa raschii* (Fig. 4). Table I gives the average length of the specimens forming the selected group on each date. It was difficult to follow maximum-number groups from the larval stages since these, when present, were always in enormous numbers. The study covered the period from July 1951 to July 1952. In the first month, there were three size-groups of *Meganyctiphanes norvegica* with larger numbers of specimens than those next to them above or below, but in subsequent months only one group predominated in numbers. It appeared to correspond to the 15 mm size-group for July 1951 and this group was, therefore, used as starting-point in the graphs (Fig. 3). The group was probably hatched in the spring of 1951. Values for specimens of 13 mm length, which MacDonald (1927) regarded as first-year spawners, were the starting-points in the graphs for *Thysanoessa raschii* (Fig. 4). In all the graphs body-weight and fat concentration were also plotted.

The information contained in these graphs can now be considered in more detail under the headings of the various characteristics studied.

**Size**

In *Meganyctiphanes norvegica* (Fig. 3) the weight increased steadily from 47 mg in July to 246 mg in December, with a corresponding increase in length from 15 to 25 mm. From January to April 1952 both weight and length remained stationary, and then increased further to 434 mg and 33 mm by July. The two main periods of growth coincided with the spring and autumn diatom outbursts.

*Thysanoessa raschii* (Fig. 4) did not grow in the autumn to the same extent as *Meganyctiphanes norvegica*. The maximum-number group remained at 13 mm and 28 mg from July 1951 until October 1951 when the weight rose to 42 mg with no further change till March 1952. The length increased to 15 mm in December and remained so until March 1952. In April the length was 17 mm and the weight 54 mg; in May and
TABLE I. AVERAGE LENGTH IN MM OF SPECIMENS IN SELECTED GROUPS OF MEGANYCTIPHANES NORVEGICA AND THYSANOESSA RASCHII PRESENTED IN FIGS. 3 AND 4.

<table>
<thead>
<tr>
<th>Date</th>
<th>M. norvegica</th>
<th>T. raschii</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. vii. 51</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>16. viii. 51</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>6. ix. 51</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>18. x. 51</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>12. xii. 51</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>16. i. 52</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>13. ii. 52</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>13. iii. 52</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>24. iv. 52</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>22. v. 52</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>19. vi. 52</td>
<td>31*</td>
<td>19†</td>
</tr>
<tr>
<td>9. vii. 52</td>
<td>33‡</td>
<td>19§</td>
</tr>
</tbody>
</table>

* Largest group was of specimens 15 mm long, but the series considered here was 1 year older and, among these, animals 31 mm long formed the largest group.
† Largest group was of specimens 7 mm long, but the series considered here was 1 year older and, among these, animals 29 mm long formed the largest group.
‡ Corresponding maximum number for series was actually in 29 mm group, but specimens were both shorter and lighter than their predecessors in the series under consideration. The larger size-group completed the series more logically.
§ Largest group was of specimens 9 mm long, but series considered here was 1 year older and, among these, animals 19 mm long formed the largest group.

June the length was 19 mm and the weight 92 and 90 mg, and in July the length remained 19 mm and the weight had increased to 99 mg. As with M. norvegica, growth occurred during the spring diatom increase.

Fat

The fat concentration of the sample of M. norvegica (Fig. 3) collected in July 1951 (6.7 %, or 3 mg/specimen) was the highest for the whole year. There was a sharp drop in August to 2.4 % (2 mg/specimen) followed by a steady rise to 16 mg/specimen (6.4 %) in October and then a slight drop in December (5.6 %, or 12 mg/specimen) and January (4.0 % or 10 mg/specimen), and an increase in February to 5.7 % (13 mg/specimen). In March the fat concentration dropped to a minimum for the year of 0.9 % (2 mg/specimen), increasing irregularly to 4.3 % (19 mg/specimen) by July 1952. Thus the general picture is one of increasing fat reserves during the autumn and spring feeding periods.

In Thysanoessa raschii (Fig. 4) the fat percentage increased from 7.0 % (2 mg/specimen) in July 1951 to 9.2 % (4 mg/specimen) in October 1951 and then steadily declined to a minimum of 1.3 % (0.5 mg/specimen) in February 1952. During the spring the fat reserves were replenished until they reached 5.2 % (5 mg/specimen) in July 1952. Autumn and spring feeding appear to have led to accumulation of fat which was used during the intermediate winter period when food was scarce.

The relatively sharp drop between February and March in Meganyctiphanes norvegica and between January and February in Thysanoessa raschii may have been associated with spawning.

Vitamin A

The content of vitamin A in Meganyctiphanes norvegica (Fig. 3) increased from 0.5 i.u./specimen in July 1951 to 1.9 i.u./specimen in September. Until April 1952 the
Fig. 3. Seasonal variations in the contents and concentrations of vitamin A and astaxanthin, oil percentage, and weight in selected groups of *Moganyctiphanes norvegica*. —, vitamin A; ———, astaxanthin; ......, weight; ———, oil.
Fig. 4. Seasonal variations in the contents and concentrations of vitamin A and astaxanthin, oil percentage, and weight in selected groups of *Thysanoessa raschii*. ——, vitamin A; ---, astaxanthin; ----, weight; ——, oil.
value fluctuated around 2.0 i.u./specimen. It then climbed steadily to 6.0 i.u./specimen in July. The vitamin A concentration of *M. norvegica* (Fig. 3) increased from 11 i.u./g in July 1951 to 13 i.u./g in September, but decreased sharply in October to 7 i.u./g. During the following months it remained around 8–9 i.u./g, and then, in April, reached its lowest level, of 6 i.u./g; thereafter it climbed steeply to 14 i.u./g in June and July. The final concentration is rather low for animals of this size (see Fisher et al., 1952).

The vitamin A content of *Thysanoessa raschii* (Fig. 4) was 0.5 i.u./specimen in July 1951 and remained at around this level until October. By December it had reached 1.3 i.u./specimen but increased no further until April, when it went up to 2.0 i.u. In May it was 2.2 i.u., regressed to 1.5 i.u. in June, and recovered to 2.2 i.u./specimen in July. The vitamin A concentration in the July 1951 sample of *T. raschii* (Fig. 4) was 20 i.u./g, and rose to 23 i.u./g in August. By September, it had fallen to 10 i.u./g and then increased, through 12 i.u./g in October to 36 i.u./g in December.

The concentration was somewhat lower in January and February but reached the December level again in March and April. Thereafter it declined to 23 i.u./g in May and to 16 i.u./g in June, climbing once more to 22 i.u./g in July 1952. As in *Meganycitiphanes norvegica* the concentrations of vitamin A in *Thysanoessa raschii* are also somewhat lower than found by us previously (Fisher et al., 1952).

**Astaxanthin**

The astaxanthin content of *Meganycitiphanes norvegica* (Fig. 3) increased 16-fold from 2 µg/specimen in July 1951 to 32 µg/specimen in July 1952 compared with a 13-fold increase in vitamin A during the same period. This increase in astaxanthin content occurred in two periods, one during the autumn months, reaching 10 µg/specimen in December, and the second from 11 µg in April to 17 µg in June, with a final steep climb to 32 µg in July. The concentration of astaxanthin in *M. norvegica* (Fig. 3) decreased from 42 µg/g in July 1951 to 29 µg/g in September, when it rose once more, reaching its highest level in February of 51 µg/g. It then steadily declined until April to 33 µg/g. Thereafter it increased to 44 µg/g by June and to 74 µg/g in July.

In *Thysanoessa raschii* (Fig. 4) the astaxanthin content in July 1951 was 0.8 µg/specimen and remained at around this level until October. It increased steadily during the next few months and by February was 2.0 µg/specimen. After a slight drop in March, the increase continued to 3.4 µg/specimen in May when the content levelled off for the rest of the period until July. Astaxanthin concentrations in *T. raschii* (Fig. 4) fluctuated during the autumn months from 27 µg/g in July 1951 to 22 µg/g in October, and then steadily increased to attain their peak in February 1952 at 50 µg/g. In March 1952 the concentration fell to 40 µg/g and decreased slightly during the next few months to 34 µg/g when the study ended in July.

**Caridea**

*Crangon allmani* was collected from Loch Fyne less regularly than the euphausiids. The results are given in Table II. Vitamin A was present, always in the ester form, in all groups except that collected in September 1951 and, when found, was chiefly, and often exclusively, in the eyes, but in concentrations much lower than in the euphausiids. The carotenoids of *C. allmani* included astaxanthin or its esters, carotenes and xanthophylls.

*C. vulgaris* (Table III) had vitamin A in all groups, confined exclusively to the eyes. The ester predominated in all those groups in which the two forms were separated. The concentrations of vitamin A in this species were usually
lower than those found in *C. allmani*, but β-carotene was found in a larger proportion of, though not in all, groups of *C. vulgaris*.

The principal carotenoid in both species of *Crangon* was astaxanthin and its concentration in the eyes, not given in Table III, was about 10 times that in the bodies, although the eyes contained only about 5% of the total carotenoids in the whole animal, the bulk of the pigments being xanthophylls.

There was no indication of any seasonal variation in either vitamin A or carotenoids in these two species of *Crangon*.

**Table II. Distribution of Oil Percentage, Vitamin A and Carotenoids per Specimen and per Gram in Whole Animal (A) and Pairs of Eyes (E) of *Crangon allmani* Collected from Loch Fyne**

<table>
<thead>
<tr>
<th>Date</th>
<th>Tissue specimens</th>
<th>Wt. (mg)</th>
<th>Oil (%)</th>
<th>Vitamin A</th>
<th>Carotenoids*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of specimens</td>
<td></td>
<td></td>
<td>i.u./specimen</td>
<td>i.u./g.</td>
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<tr>
<td>6. ix. 51 A 17</td>
<td>403</td>
<td>2.1</td>
<td>0</td>
<td>0.6</td>
<td>2.9</td>
</tr>
<tr>
<td>E 17</td>
<td></td>
<td>1.6</td>
<td>0</td>
<td>0</td>
<td>1.1</td>
</tr>
<tr>
<td>18. x. 51 A 11</td>
<td>260</td>
<td>4.2</td>
<td>0.28</td>
<td>1.1</td>
<td>4.2</td>
</tr>
<tr>
<td>E 11</td>
<td></td>
<td>2.9</td>
<td>0.15</td>
<td>150</td>
<td>1.8</td>
</tr>
<tr>
<td>13. iii. 52 A 18</td>
<td>1109</td>
<td>1.8</td>
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<tr>
<td>E 18</td>
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<td>5.4</td>
<td>0.14</td>
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<tr>
<td>22. iv. 52 A 10</td>
<td>514</td>
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<td>E 10</td>
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<td>829</td>
<td>0.7</td>
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<tr>
<td>E 71</td>
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<td>1.1</td>
<td>0.11</td>
<td>27</td>
<td>—</td>
</tr>
</tbody>
</table>

* β-carotene absent.

**Astacura**

*Nephrops norvegicus* was collected in small groups from Loch Fyne during the visits made to collect euphausiids between July 1951 and July 1952. The animals were dissected and various organs were analysed in separate groups. The results are given in Table IV. From February 1952, the specimens were separated into eyes, gizzard, hepatopancreas and rest of body. As previous experience had shown that this last portion contained little or no vitamin A, expenditure of large volumes of solvents for the extraction of the large bulk of material in a number of bodies seemed unnecessary and to study any seasonal variation we confined our attention to the organs mentioned separately above.

In the group collected on 14 August 1951 the eye-stalks were separated from the eyes to determine whether the vitamin A in the eyes was associated with the sinus gland of the eye-stalk, but all the vitamin was in the eyes themselves. In contrast to the shrimps, the carotenoid pigment in the eyes was in concentrations of a similar order to those in the rest of the body and was exclusively astaxanthin. Carotenes and xanthophylls were present with astaxanthin in the other parts of the animals and small measurable amounts of β-carotene were found in some groups, usually in the hepatopancreas. There
<table>
<thead>
<tr>
<th>Date</th>
<th>Locality</th>
<th>No. of specimens</th>
<th>Av. wt. (mg)</th>
<th>Oil (%)</th>
<th>Vitamin A</th>
<th>Total carotenoids</th>
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</thead>
<tbody>
<tr>
<td>21. viii. 51</td>
<td>Plymouth</td>
<td>93</td>
<td>680</td>
<td>11</td>
<td>0.05</td>
<td>5.2 (g)</td>
</tr>
<tr>
<td>19. ix. 51</td>
<td>Plymouth</td>
<td>52</td>
<td>1055</td>
<td>0.8</td>
<td>0.05</td>
<td>2.3 (g)</td>
</tr>
<tr>
<td>27. xi. 51</td>
<td>Colwyn Bay</td>
<td>161</td>
<td>1176</td>
<td>1.2</td>
<td>0.08</td>
<td>4.9 (g)</td>
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<tr>
<td>12. xii. 51</td>
<td>Abergele</td>
<td>164</td>
<td>1239</td>
<td>1.0</td>
<td>0.06</td>
<td>4.2 (g)</td>
</tr>
<tr>
<td>2. i. 52</td>
<td>Conway</td>
<td>148</td>
<td>1360</td>
<td>1.4</td>
<td>0.06</td>
<td>6.2 (g)</td>
</tr>
<tr>
<td>16. i. 52</td>
<td>Conway</td>
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<td>873</td>
<td>1.6</td>
<td>0.10</td>
<td>2.9 (g)</td>
</tr>
<tr>
<td>16. i. 52</td>
<td>Conway</td>
<td>92</td>
<td>568</td>
<td>1.4</td>
<td>0.06</td>
<td>1.8 (g)</td>
</tr>
<tr>
<td>26. i. 52</td>
<td>Colwyn Bay</td>
<td>79(a)</td>
<td>1819</td>
<td>0.9</td>
<td>0.07</td>
<td>8.1 (g)</td>
</tr>
<tr>
<td>26. i. 52</td>
<td>Colwyn Bay</td>
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</tr>
<tr>
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<td>0.08</td>
<td>6.7 (g)</td>
</tr>
<tr>
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</tr>
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<tr>
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<td>Pendwffyn</td>
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<tr>
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<td>Pendwffyn</td>
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<tr>
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<td>Pendwffyn</td>
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<td>1105</td>
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<td>0.04</td>
<td>5.5 (g)</td>
</tr>
</tbody>
</table>

(a) 'Berried'♀. (b) Mixed ♂ and ♀ without eggs.
TABLE IV. DISTRIBUTION OF OIL PERCENTAGE, VITAMIN A PER SPECIMEN AND PER GRAM, CAROTENIODS PER SPECIMEN, PER GRAM AND PER GRAM OIL, AND \( \beta \)-CAROTENE PER GRAM OIL IN *NEPHROPS NORVEGICUS* FROM THE CLYDE SEA AREA

For eyes and e' e-stalks all values are per pair

<table>
<thead>
<tr>
<th>Date</th>
<th>Organ</th>
<th>No. of specimens</th>
<th>Av. wt. (g)</th>
<th>Oil (%)</th>
<th>Vitamin A</th>
<th>Total carotenoids</th>
<th>( \beta )-carotene, ( \mu )g/g oil</th>
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</thead>
<tbody>
<tr>
<td>3. vii. 51</td>
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</table>

For eyes and e' e-stalks all values are per pair.
was no consistent seasonal variation in the concentrations of vitamin A, carotenoids or fat in any of the organs investigated. The hepatopancreas was much richer in fat than the other parts of the animal.

Of *Homarus vulgaris*, only groups of eyes were analysed and the results are shown in Table V. Concentrations of both vitamin A and carotenoids, the latter predominantly astaxanthin with a little xanthophyll, were fairly high during the early months of 1951, but by July the vitamin A level had dropped to a value from which it deviated little during the remaining period of investigation up to April 1952. The content and concentration of carotenoids was fairly uniform throughout 1951, but in January 1952 it dropped to about half the previous level and persisted so in the following months until April.

### Table V. Oil Percentage and Vitamin A and Carotenoids per Pair and per Gram in the Eyes of *Homarus vulgaris* of Unknown Origin

<table>
<thead>
<tr>
<th>Date</th>
<th>No. of eyes</th>
<th>Wt. (mg/pair)</th>
<th>Oil (%)</th>
<th>Vitamin A</th>
<th>Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>i.u./pair</td>
<td>i.u./g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>µg/pair</td>
<td>µg/g</td>
</tr>
<tr>
<td>15. i. 51</td>
<td>57</td>
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<td>12</td>
<td>17</td>
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<td>0·7</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
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<td>6·5</td>
<td>9·1</td>
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<td>3·7</td>
<td>6·2</td>
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<td>6·1</td>
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### Discussion

The foregoing observations indicate that, whereas the fat, vitamin A and astaxanthin reserves of the euphausiids varied markedly with the season, no such changes occurred in the shrimps, *Crangon allmani* and *C. vulgaris*, or in *Nephrops norvegicus*. Vitamin A and carotenoids in the eyes of *Homarus vulgaris* varied to some extent with the season. These eyes weighed between 0·5 and 0·8 g per pair and were all preserved, stored and analysed in the same way, but the origin of the lobsters was unknown and may have varied through the year. This might account for the absence in 1952 of the trend observed in the previous year, when vitamin A and carotenoids increased during the spring to a maximum in April.

An interesting comparison between the two species of *Crangon* is noteworthy. The concentration of vitamin A in the eyes of *C. allmani* was higher than in those of *C. vulgaris*. The difference may be associated with the relative visual requirements of the two animals, as *C. allmani* lives at much greater depths and, therefore, in much darker surroundings than *C. vulgaris*, although
both are benthic animals in which vision may be comparatively unimportant. On the other hand, it is possible that *C. allmani* from Loch Fyne also inhabited by large populations of vitamin A-rich euphausiids may have, by feeding on their living or dead bodies, accumulated its reserves at a faster rate than *C. vulgaris* from an environment where its food contains much less vitamin A. Admittedly, though *C. allmani* is carnivorous, we do not know whether it in fact preys on euphausiids. Another possibility is that both obtain their vitamin A by the conversion of a precursor such as \( \beta \)-carotene and that the process operates more efficiently in *C. allmani*, which usually had no \( \beta \)-carotene, than in *C. vulgaris* which contained it in most parts of the body.

We have established for a considerable range of sizes that the presence of the bulk of the vitamin A and of at least half the total astaxanthin in the eyes of both *Meganyctiphanes norvegica* and *Thysanoessa raschii* is a characteristic independent of the season. We have confirmed our earlier findings (Kon & Thompson, 1949a; Fisher *et al.*, 1952) that euphausiid vitamin A occurs mainly in the ester form, but we now know that this form predominates only in the eyes. Ester and alcohol were usually present in about equal amounts in the small quantity of vitamin A found in the bodies.

The ester is the form of vitamin A stored in the liver by vertebrates and the alcohol is the active form passed to the rest of the body in the blood stream. Thus most of the vitamin A in the euphausiid eye is in the storage form with only a small amount in the active state.

In the studies reported here and previously (Fisher *et al.*, 1952, 1953), we found the concentration of vitamin A in the eyes of *Meganyctiphanes norvegica* to be within the range of 2000–20,000 i.u./g dry weight and even if 95% of this is ester, the concentration of vitamin A alcohol would be between 100 and 1000 i.u./g. Such quantities would certainly be adequate for visual purposes, since, as we have previously pointed out (Fisher *et al.*, 1952), Wald (1935) found that in mammalian retinas the concentration of vitamin A adequate for vision is about 70 i.u./g dry weight and in frog retinas about 1200 i.u./g, and Morton & Rosen (1949) recorded a maximum value for whole eyes of frogs equivalent to 300 i.u./g dry weight.

Retinene (vitamin A aldehyde) which participates in the visual cycle of higher animals (Wald, 1945) is absent. Thus vitamin A in the eyes of euphausiids is apparently in excess of that required for vision. What, therefore, is its function, if any? In Crustacea, the hepatopancreas is a digestive organ and not a storage organ like the vertebrate liver. Both vitamin A ester and astaxanthin are found in the euphausiid eye in much higher concentrations than in the eyes or bodies of other animals and there is no obvious function for these large quantities. It may be that they are no more than metabolic end-products accumulating there during the life of the euphausiids, like vitamin A and carotenoids in, for example, fish livers (Macpherson, 1933).

Figs. 3 and 4 show that *M. norvegica* and *Thysanoessa raschii* accumulated
vitamin A and astaxanthin mainly during the autumn and spring when diatom outbursts stimulated feeding. In *Meganyctiphanes norvegica* the concentrations of vitamin A reached maxima in June–July and in September, falling during the winter when growth continued but the vitamin A was not being accumulated. The astaxanthin concentration remained fairly high all winter in *M. norvegica*, dropping with that of vitamin A in April, a month after the fat concentration reached its minimum. Thus there was a time-lag between the resumption of growth at the faster spring rate, which probably began between February and March in *M. norvegica* and between January and February in *Thysanoessa raschii*, and an increase in the fat reserves, which began a month later, and another month passed before the contents of vitamin A and astaxanthin began to increase. Spawning may have occurred at around this time and depleted the stocks of fat, vitamin A and astaxanthin.

*Meganyctiphanes* began accumulating vitamin A and astaxanthin again in April (Fig. 3), whereas in *Thysanoessa* the concentration of vitamin A did not begin to rise until June or July (Fig. 4) and, even then, that of astaxanthin was still falling.

The difference between the two species may possibly be explained in terms of their food. *Meganyctiphanes norvegica* of the size considered here would be carnivorous (Macdonald, 1927), whereas the smaller *Thysanoessa raschii* would probably be mainly herbivorous, although less is known about the feeding of this species. Gillam, el Ridi & Wimpenny (1939) found that both phytoplankton and zooplankton reached their highest growth in May and August but the plant concentrations were much more transitory than those of animals. Zooplankton densities remained fairly high for 2 or 3 months after the peaks, whereas those of the phytoplankton soon became much lower. *Meganyctiphanes norvegica* might thus have a continued supply of adequate animal food but *Thysanoessa raschii* would have to subsist on rapidly diminishing stocks of plants.

Further evidence of a difference in feeding habits between *Meganyctiphanes norvegica* and *Thysanoessa raschii* is that the former (Fig. 1) accumulated astaxanthin much more rapidly than vitamin A, during spring 1951, whereas in *T. raschii* (Fig. 2) the two substances increased at a more closely similar rate. A greater proportion of zooplankton, composed predominantly of copepods and thus rich in astaxanthin, in the diet of *Meganyctiphanes norvegica* would account for its increased stores of this carotenoid. Another explanation may be found in the larger pigmented areas of the body in *M. norvegica* than in *Thysanoessa raschii*. Our analytical results have shown that a much higher percentage of the total astaxanthin is in the eyes, and, therefore, less is in the bodies, of *T. raschii*, than in those of *Meganyctiphanes norvegica*, which, therefore, requires more astaxanthin for its pigmentation.

In neither species of euphausiid was the accumulation of vitamin A and astaxanthin as marked in the spring of 1952 as in the previous year. The
lower concentrations of these substances found during the winter months of 1951–52 (Figs. 1, 2) may, in some way, have been associated with the absence from our hauls of very large specimens of *M. norvegica* and the reduction in the numbers of both this species and *Thysanoessa raschii* during these months and the following spring. It is interesting that, in February 1952, fishermen at Monaco were complaining that *Meganyctiphanes norvegica* appearing in the port were smaller than usual at that time of year.

Examination of Fig. 4 shows that in *Thysanoessa raschii* there was a marked inverse relationship between the concentrations of fat and vitamin A through the year, and a similar relationship was also detectable in *Meganyctiphanes norvegica* (Fig. 3). The feeding habits of the animals probably account for the phenomenon. Fig. 4 indicates that *Thysanoessa raschii* lived mainly on its reserves during the period from October to March. Body-weight remained fairly constant but the stores of fat were reduced considerably. Continued accumulation of vitamin A and astaxanthin either from food eaten or by conversion from a precursor within the body resulted in increased concentrations of these substances. With the resumption of large-scale feeding in the spring, growth was more rapid and fat was again stored with the result that the concentrations of vitamin A and astaxanthin fell as these accumulated less rapidly than fat owing to the time-lag discussed above. Cause and effect are less easily elucidated in *Meganyctiphanes norvegica*, but there is no doubt that the inverse relationship is also found in this species. Gillam *et al.* (1939) noted a similar inverse relationship in their study between the vitamin A concentration and fat-free solids of gross plankton over the period of a year, apart from the spring increase when vitamin A increased as well. They suggested that the relationship was indicative of the type of behaviour expected from an autocatalyst of growth. It may, however, merely be a measure of the proportions in the total plankton of various planktonic organisms. At the peak periods for fat-free solids, the plankton would be composed mainly of plants and smaller Crustacea, such as copepods which, we know, lack vitamin A. This mixed population would be grazed down by larger animals, especially fish larvae and euphausiids, which form or accumulate vitamin A and concentrate it in their bodies. The population density of these organisms would be relatively much smaller than that of copepods and diatoms, so that the concentration of fat-free solids would be reduced, especially as most of the diatoms die and sink to the bottom soon after their outbursts, thereby further reducing the solid material present.

All our evidence points to the richness in vitamin A of euphausiids in comparison with other Crustacea. In Loch Fyne, *Meganyctiphanes* and *Thysanoessa* were taken in the same hauls as *Calanus finmarchicus* (Gunnerus) and *Euchaeta norvegica* Boeck and very often, near the bottom, with *Crangon allmani* and *Pandalus bonnieri* Caullery. Neither the copepods nor the decapods contained vitamin A in amounts approaching those in the euphausiids. The
question arises what source of the vitamin or its precursors is available to these animals and closed to their neighbours. We believe that our study may have shed a little light on the problem. An examination of the monthly graphs for *Meganyctiphanes* in Fig. 1 shows that the highest concentrations of vitamin A and astaxanthin were found in the smallest and largest specimens, although the content of both vitamin A and astaxanthin increased throughout life. It seems very likely that in the former the vitamin and the pigment are passed on to the larva from the egg. But why do the concentrations increase steeply in animals over 30 mm long? This size is not associated with the achievement of maturity, since adult animals are found upwards of 20 mm long. The high concentrations were observed in large specimens at all times of year and so were not connected specifically with the breeding period.

*Thysanoessa raschii* showed certain of the trends noted in *Meganyctiphanes norvegica*. The increase in vitamin A and astaxanthin concentrations usually appeared in animals between 15 and 20 mm long. Again these are not related to the attainment of sexual maturity which occurs in this species at about 13 mm length (Macdonald, 1928).

We have examined the gut contents of *Meganyctiphanes norvegica* of different sizes. Smaller animals (15–25 mm long) eat much detritus of vegetable origin, together with diatoms, of which fragments were found in the gut. In the larger specimens (over 30 mm long), detritus was also present, but accompanied there by crustacean fragments, mainly of copepod origin and often identifiable as parts of *Calanus finmarchicus* and *Euchaeta norvegica*. Macdonald (1927) made a more systematic investigation of the food of *Meganyctiphanes norvegica* and reached the following conclusions:

‘(1) Organic detritus is eaten most abundantly during the first months of the year; (2) *Meganyctiphanes*, ranging from 21–29 mm, feed more extensively on vegetable detritus than do larger or smaller specimens (it should be noted that this size was by far the most abundant in the Clyde Sea area); (3) Copepods are eaten most extensively by the larger specimens viz. 31–39 mm and (4) the smaller specimens, 13–19 mm, feed most extensively on diatoms and “wet dust”).

‘Wet dust’ was also called, by Macdonald, flocculent detritus and consisted of a mass of greenish brown unidentifiable particles, including shells of diatoms and peridinians, spores of algae and possibly argillaceous particles. The species of diatoms he found most abundantly in *M. norvegica* were *Paralia sulcata* (Heiberg) Cleve, *Thalassiosira nordenskiöldi* Cleve, *T. gravida* Cleve and *Coscinodiscus* spp. Other diatoms occasionally eaten in quantities included *Nitzschia* and *Skeletonema*. We have analysed pure cultures of several diatoms, including *Thalassiosira gravida* Cleve, *Coscinodiscus concinnus* W. Sm., *Phaeodactylum tricornutum* Bohlin, and *Skeletonema costatum* (Grev.) Cleve and the dinoflagellate, *Peridinium trochoideum* (Stein) Lemm., and found no vitamin A in any of these organisms.
Little is known about the feeding of *Thysanoessa raschii*. Our own experience has been to find the gut empty except for occasional detritus or other unidentifiable material. It is difficult to postulate a change of diet, as in *Meganyctiphanes norvegica*, in animals so much smaller when the concentrations of vitamin A and astaxanthin begin to rise, unless the larger prey on larval copepods. We have never found, nor did Macdonald (1927), parts of euphausiids in the guts of others, so that it is very unlikely that larger euphausiids obtain their vitamin A preformed by eating smaller ones.

The change-over from mainly vegetable to mainly animal food, observed by both Macdonald and ourselves in specimens of *M. norvegica* over 30 mm long, might well be the cause of their increased rates of storage of vitamin A and astaxanthin, resulting from differences in the carotenoid constituents in the diet. The increased uptake and storage of astaxanthin would be thus explained because this pigment is the principal carotenoid in copepods. Vitamin A and astaxanthin are both absent, however, from diatoms and there is no vitamin A in *Calanus finmarchicus* (Euler, Hellström & Klussmann, 1934; Lederer, 1938; Fisher *et al.*, 1952), and only rarely in *Euchaeta norvegica* (Fisher *et al.*, 1952). Direct uptake of both substances is thus unlikely to account for their presence in the young herbivorous stages of *Meganyctiphanes*, and that of vitamin A for its presence in larger predominantly carnivorous animals. We must, therefore, look for precursors that these euphausiids can convert to astaxanthin and vitamin A. The plant food of the herbivores contains β-carotene, a known provitamin A and possibly also a precursor for astaxanthin. Neither β-carotene nor any other known provitamin A is found in more than trace amounts in the copepods and yet the larger stages of *M. norvegica* feeding on them appear capable of storing vitamin A much more rapidly than those living on plant material. They must, therefore, utilize as precursors either known carotenoids or other substances, normally not regarded as vitamin A precursors.

Let us consider the carotenoids present in the plankton organisms involved in these food-chains. The principal carotenoids in diatoms are β-carotene and xanthophylls, including certain pigments peculiar to these plants, such as diatoxanthin (Strain, Manning & Hardin, 1944). This carotenoid has an absorption spectrum very similar to that of zeaxanthin which, according to Goodwin (1952), is probably 3:3′-dihydroxy-β-carotene. Goodwin believes that diatoxanthin may be a cis-isomer of zeaxanthin and so possess the same structural formula. Astaxanthin is 3:3′-dihydroxy-4:4′-diketo-β-carotene (Kuhn & Sørensen, 1938), so that zeaxanthin, and, therefore, probably diatoxanthin, are structurally intermediate between β-carotene and astaxanthin. The oxidation of β-carotene to astaxanthin, if it occurs at all, may thus go as far as diatoxanthin in the diatoms and be completed in the zooplankton, giving rise to astaxanthin and vitamin A in euphausiids and to astaxanthin only in copepods. Zeaxanthin itself is inactive as a provitamin A for pigs (Braude *et al.*, 1941), but invertebrate carotenoid metabolism may be different,
and it is interesting to note that recently Lenel (1953) found in the organs of the shore-crab, *Carcinus maenas* (Pennant), not only β-carotene and astaxanthin or their stereoisomers but also more or less oxidized pigments intermediate between these two. It is generally believed that Crustacea are unable to synthesize astaxanthin *de novo* (Sørensen, 1936; Fox, 1947). Fox suggested that pigments similar to astaxanthin may be formed in coelenterates, sponges, molluscs, echinoderms and other invertebrates, and that the common occurrence of acidic or acidogenic carotenoids among many invertebrate animals indicates a unique ability to effect a partial oxidation of commoner polyene molecules without actually splitting them. However, the only carotenoid molecule available in any quantity in its copepod diet to *Meganyctiphanes norvegica* is astaxanthin. If *Meganyctiphanes* converts this pigment to vitamin A, a reduction is involved but, as Fox (1953) points out, the general tendency in the animal body is towards the oxidation of carotenoids rather than to their reduction. The presence of oxygen in the β-ionone rings of carotenoids has always been considered by biochemists as a bar to their conversion to vitamin A, although Goodwin (1951) has suggested that this criterion may have to be modified since the work of Karrer, Jucker, Rutschmann & Steinlin (1945) on the production of carotenoid epoxides indicates that 5:6 epoxides, e.g. 5:6-5':6'-diepoxy-β-carotene, are vitamin A precursors. As Goodwin pointed out, the formation of vitamin A may occur after initial conversion of the epoxides to β-carotene, in other words, after their reduction.

Our evidence certainly suggests that euphausiids utilize a vitamin A precursor not available to other Crustacea living around them, and it is difficult to ignore the coincidence between the appearance of astaxanthin in their diet and the appreciable increase in their vitamin A reserves.

The suggestion that astaxanthin may be a precursor for vitamin A is not entirely new; Drummond & MacWalter (1935) thought that astacin from krill might be converted to vitamin A by the whale. We now know (Kon et al., 1949a, b) that there is no need for such a hypothesis since the vitamin is already present preformed in krill; Morton (1940) believed it possible that certain carnivorous animals, such as fishes, may be able to use astaxanthin from Crustacea in their food as a basis for conversion to vitamin A; and more recently Collins, Love & Morton (1953) have suggested astaxanthin as a possible precursor for vitamin A₂. In dealing with possible sources of vitamin A for euphausiids a further factor must be considered. Detritus is eaten by euphausiids of all sizes and is, therefore, unlikely to account for increased accumulation of vitamin A in larger euphausiids. Nevertheless, the importance of detritus as a source of carotenoids must not be overlooked (see Fox, 1950). Fox, Isaacs & Corcoran (1952) have estimated that in the waters off the coast of California living cells form only 1·5–4% of the total colloidal or otherwise finely particulate organic matter, called leptopel, and its carotenoid
content is almost unknown. Earlier, Fox (1937) filtered off detritus and microplankton from 4000 l. of sea water and extracted from the deposit 0.1 mg of xanthophylls and 0.02 mg of carotenes. Marine muds are also rich in carotenoids (Fox, Updegraff & Novelli, 1944), especially carotenes. This part of their diet could, therefore, provide by conversion at least part of the vitamin A found in euphausiids.

Be it as it may, Euphausiacea seem unique in their ability to accumulate vitamin A in the sea and the interesting possibility remains that they, of all Crustacea, may be able to utilize the ubiquitous crustacean pigment, astaxanthin, as a precursor of vitamin A. The joint presence in the euphausiid eye of large amounts of vitamin A and astaxanthin in similar proportions in different size-groups, as indicated in Figs. 1 and 2, strongly suggests a close interrelationship of these substances in the carotenoid metabolism of euphausiids.

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**SUMMARY**

Results are given for measurements of fat, vitamin A and carotenoids in groups of Meganyctiphanes norvegica, Thysanoessa raschii, Crangon allmani, C. vulgaris, Nephrops norvegicus and eyes of Homarus vulgaris, collected at regular intervals for a year or longer.

In both species of euphausiids vitamin A was consistently present mainly in the ester form and concentrated chiefly in the eyes. More than half the total astaxanthin was in the eyes. No other carotenoids were detected.

Astaxanthin and vitamin A were accumulated by the euphausiids more rapidly during the spring and autumn-feeding periods associated with diatom outbursts than at other seasons. There was a delay in Thysanoessa raschii, but not in Meganyctiphanes norvegica, between resumption of more rapid growth during spring and autumn and increase in concentration of vitamin A and astaxanthin. The difference was possibly due to differences in diet of the two species in relation to the seasonal biological composition of the plankton.

In the euphausiids, concentrations of vitamin A and astaxanthin were much
higher in larvae and in adults over 30 mm long of M. norvegica, and in larvae and adults over 15 mm of Thysanoessa raschii, than in mature adults of Meganyctiphanes norvegica of 20–30 mm and Thysanoessa raschii of 13–15 mm, respectively. An inverse relationship between fat and vitamin A concentration was noted in both species throughout the year.

In Crangon spp. and Nephrops norvegicus there was no evidence of any seasonal variation of either vitamin A or carotenoids. Differences in the carotenoid metabolism of the two species of Crangon are discussed.

In the eyes of Homarus vulgaris, vitamin A and carotenoids reached their highest concentrations in the spring, gradually decreasing during the rest of the year.

The metabolism of vitamin A and carotenoids in euphausiids and other Crustacea is considered. The origin of the large reserves of vitamin A in the eyes of euphausiids is discussed, and special attention is given to the importance of astaxanthin as a possible precursor.

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