A complete life cycle of the calycophoran siphonophore *Muggiaea kochi* (Will) in the laboratory, under different temperature conditions: ecological implications

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SUMMARY

For the first time, a complete life cycle of a siphonophore has been achieved in culture at different temperatures, in the small species *Muggiaea kochi*. The larval, polygastric and eudoxid stages are described, and their duration and rate of production determined. The results show that the cycle from egg to egg lasts two weeks at 24 °C and three weeks at 18 °C. Furthermore, two types of life cycle have been observed: a short cycle at 18 °C and 24 °C characterized by a short (2–3 weeks) polygastric phase, and a long cycle at 13 °C marked by the persistence of a stationary polygastric phase. The experimental results are discussed in relation to the seasonal distribution of the species in the Bay of Villefranche-sur-mer.

1. INTRODUCTION

Siphonophores are marine pelagic Cnidaria, the colonies of which vary from several millimetres to several metres in length according to the species and the age of the colony. They are very abundant, and are one of the major carnivorous groups of the plankton. However, despite their abundance the study of their populations poses several problems. First, the colonies are very fragile and only the swimming nectophores or reproductive gonophores, and sometimes the bracts, are found in plankton samples, giving only very limited information about the stage of development of the colonies and their capacity for predation, linked to gastrozooid number. Secondly, moreover, in diphyid calycophores, which represent the majority of siphonophore species, there are two types of specimens: the first type are polygastric colonies, where the distal extremity of the stolon fragments to release monogastric sexual colonies or eudoxids, the second type. The eudoxids live autonomously, themselves budding several successive gonophores. Once each gonophore has liberated its gametes, it is detached, and it degenerates while a new one develops.

Knowledge of generation time, the lifespan of colonies, the duration of eudoxid liberation by these colonies, as well as the number of successive gonophores that may be differentiated by a single eudoxid, evidently is necessary to understand the numerical data obtained from counting nectophores and gonophores in plankton samples. We have defined these parameters for the small diphyid calycophore *Muggiaea kochi* raised in culture at three different temperatures.

This species is one of the most easily sampled siphonophores in plankton tows, owing to its small size and abundance. It is very common in the Mediterranean plankton, and has a widespread geographical distribution, having been found at numerous neritic sites in the Atlantic Ocean (Mackie *et al.* 1987). Periodic seasonal and interannual fluctuations of *M. kochi* and of the closely related species *M. atlantica* have been used as indicators of water mass movements (Corbin 1947; Southward 1962). Moreover, in the waters of the Bay of Villefranche-sur-mer, up to 1981, only *M. kochi* was abundant in the spring. Since 1982, there have been, first of all, simultaneous blooms of the two species in the same water masses (*M. atlantica* hitherto having been found only exceptionally), and then, since 1987, *M. atlantica* has predominated over *M. kochi* (C. Carré & D. Carré, unpublished observations). To explain the recent co-existence of the two species, followed by the predominance of *M. atlantica*, will require a study of their spatio-temporal distribution in relation to hydrographical conditions and, in addition for *M. atlantica*, a study of the biological parameters here defined for *M. kochi*.

2. MATERIAL AND METHODS

Muggiaea kochi (Will) occurs almost year-round in the Bay of Villefranche-sur-mer, and reaches maximum abundance during May and June and sometimes also in autumn. Male and female eudoxids were collected in plankton tows between 0–50 m in June. Female gametes (liberated at the ovule stage) were fertilized by adding male gametes released from cutting up mature male gonads, and then placed in culture at 18 °C. Two days after fertilization, the young calyconula larvae were placed in lots of 100, at three different temperatures: 13 °C, 18 °C and 24 °C, and in alternating 12 h light–12 h dark cycle. The water in the cultures was renewed daily by picking out the

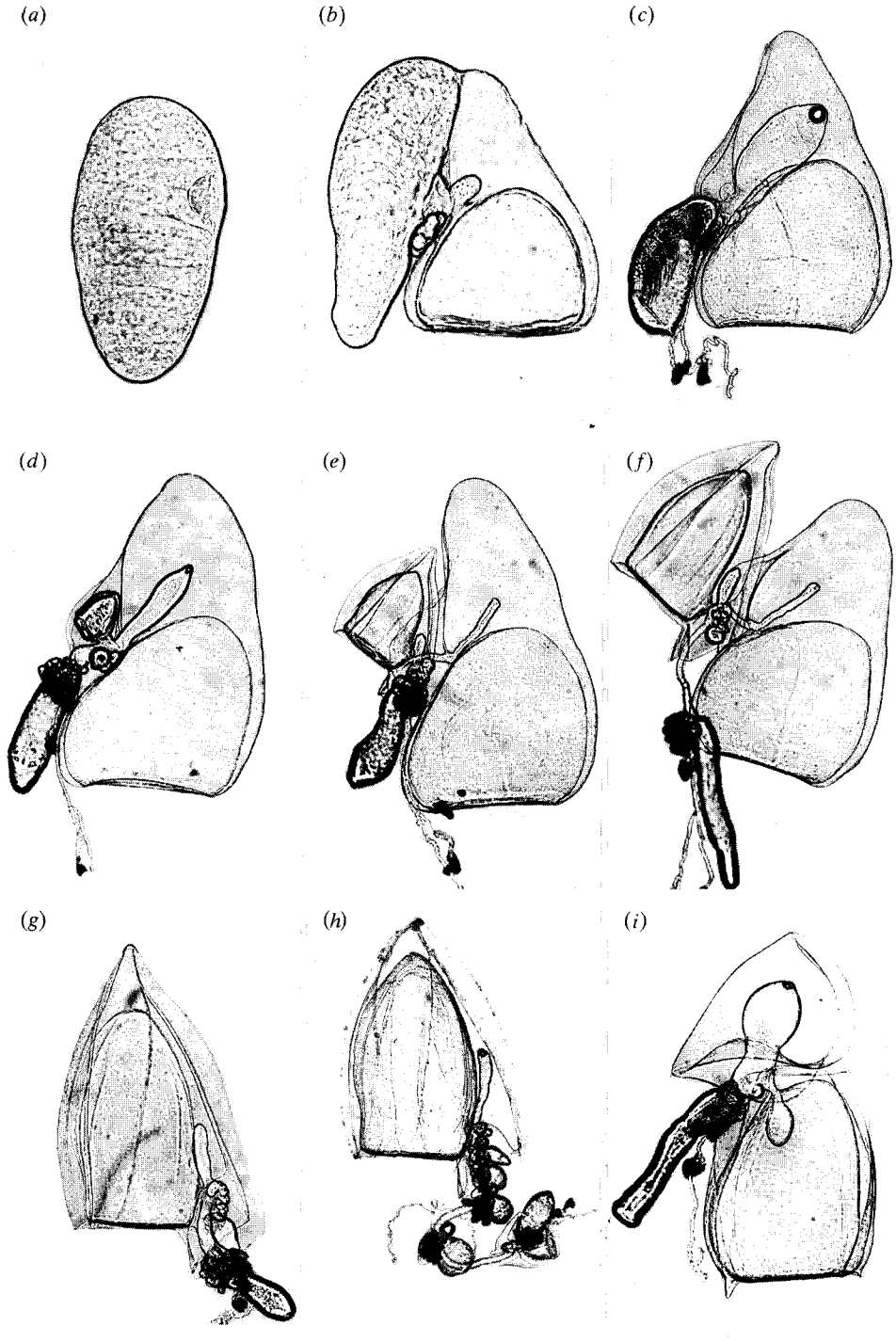

Figure 1. Principal stages of development of *Muggiaea kochi*: (*a*) planula larva; (*b*) young calyconula larva with the rudiment of the larval nectophore, the larval stem, the tentacle and the beginning of the gastric cavity; (*c*, *d*) calyconula larvae showing the rapid growth of the larval nectophore and at the same time, mobilization of the reserves of the embryonic body with the differentiation of its distal region into the gastrozooid; (*e*) calyconula larva showing the first cormidium with well-differentiated gastrozooid and tentacle, and the beginning of stolon formation with a bud and the differentiation of the second nectophore (the definitive one); (*f*) stage with two nectophores showing the rapid growth of the definitive nectophore and the elongation of the stolon with the rudiment of the second cormidium; (*g*) young colony with a single nectophore just after the loss of the larval nectophore; (*h*) adult colony reared in culture liberating eudoxids; (*i*) young eudoxid from culture at the time of liberation.

specimens. Microzooplankton collected from the sea and filtered through 50 μm mesh was provided as food for the larvae, and subsequently for the colonies and eudoxids. Each day the number of larvae, colonies and eudoxids was counted, and growth was followed by measurements *in vivo* and from photomicrographs.

3. RESULTS

(*a*) *Larval and post-larval development* (*figure 1*)

The development of *Muggiaea kochi* is largely unknown, although Chun (1897) described and figured several stages. The different phases, however, are very

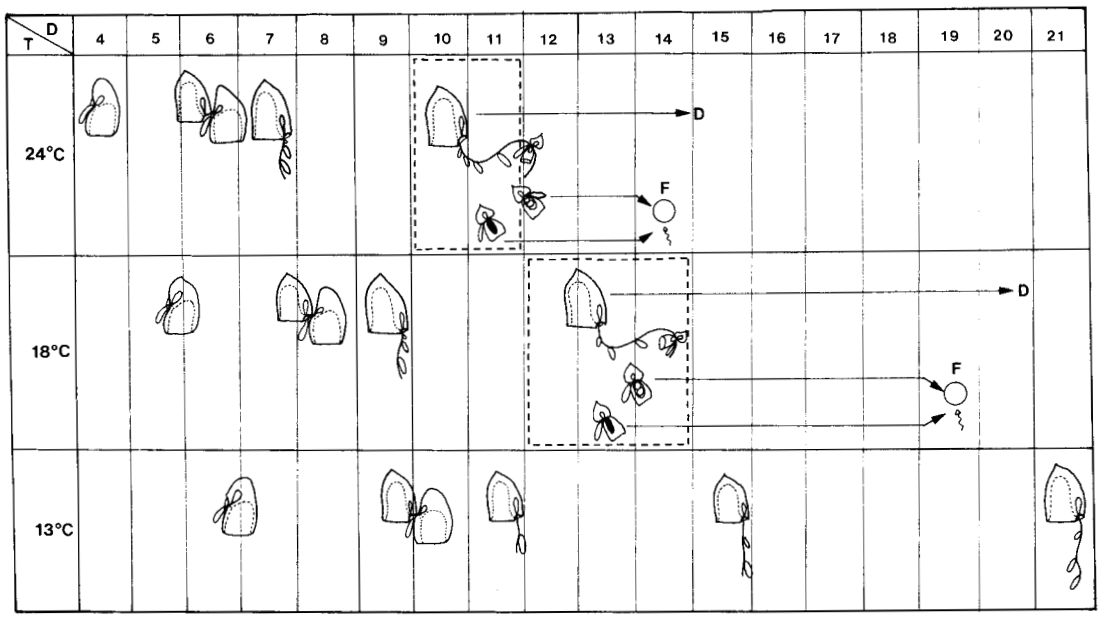

Figure 2. Daily appearance of the different rudiments and of eudoxid liberation of *Muggiaea kochi*, as a function of temperature. D, death; F, fertilization.

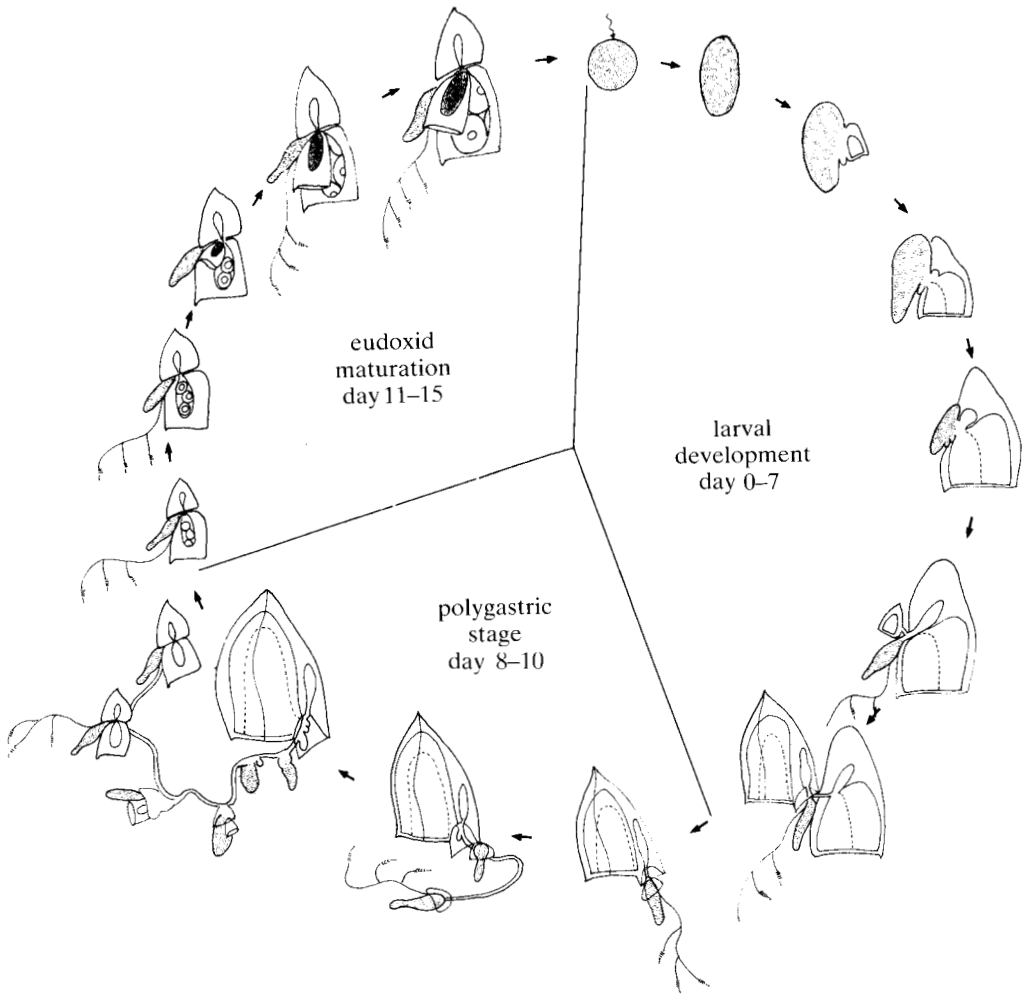

Figure 3. Diagram of complete developmental cycle of *Muggiaea kochi* with the duration of the larval, polygastric and eudoxid phases at 24 °C.

similar to those of the closely related species *Muggiaea atlantica*, as described by Russell (1938), and to those of other diphyids (Carré 1967; Carré 1979). Cleavage is at first total and equal, then partial and superficial, and results in the planula stage (figure 1*a*). The presence of a sheet of endoderm, arising by de-

lamination on one face of the planula, produces a bilateral symmetry that is more pronounced at the calyconula stage with the development of the larval nectophore. Differentiation and growth of the larval nectophore (figure 1 *b*, *c*) is rapid, but varies according to temperature. A second nectophore rudiment, which gives rise to the single definitive adult nectophore, appears on the stalk linking the larval nectophore to the remainder of the calyconula (figure 1 *d*, *e*). This stalk represents the future stolon of the colony. At the same time, the primary gastrozooid, which has differentiated from the posterior region of the planula, becomes functional with the opening of its mouth. Laterally, a diverticulum gradually colonized by cnidocytes, differentiates on the ventral face of the larva and forms the primary tentacle (figure 1 *c*).

The post-larval stage, with two functional nectophores, is very short (figure 1,*f*). After several hours, the larval nectophore is detached from the remainder of the colony after the reserves of its somatocyst have been withdrawn. A young colony of *M. kochi* results, whose further development is marked by a gradual growth of the nectophore, and by the budding, of new organs (gastrozooid and tentacle, bract and gonophore, arranged into cormidia) on the proximal part of the stolon (figure 1 *g*, *h*). The latter eventually are liberated from the end of the stolon as eudoxids (figure 1 *i*). In our cultures, the morphology of the different stages in larval and post-larval development were identical whatever the temperature.

(*b*) *Colony growth and eudoxid liberation as a function of temperature* (*figure 2*)

In the Bay of Villefranche-sur-mer, *M. kochi* is usually abundant in May and June when the surface water temperature is around 18 °C–20 °C. Both its polygastric and monogastric (eudoxid) forms are present. In our cultures, we have followed the appearance of different larval structures, colony growth and eudoxid liberation, at three different temperatures, namely 13 °C, 18 °C and 24 °C. The results are summarized in figure 2.

(i) 13 °C

Larval and post-larval development, the end of which is marked by the loss of the larval nectophore, lasts on average 11 days, and gives rise to young colonies of small size. In these, the stolon elongates comparatively slowly (one functional gastrozooid by day 14 and two by day 15) and does not liberate eudoxids. Despite the high mortality at the beginning of the experiment (50 specimens by day 8), there were still 26 specimens of the first generation at day 21 when the experiment stopped.

(ii) 18 °C

Post-larval development ended at day 9. The growth of the colony was first of all marked by the elongation of the stolon; on average there were three functional gastrozooids at day 10 and five at day 11. Between days 12 and 14, stolon growth was accompanied by eudoxid release. After 14 days, the stolon regressed and at day 21 all the specimens of the first generation were dead. Of the 100 calyconulas at the beginning of the experiment, 79 grew to be young colonies and these liberated 110 eudoxids.

(iii) 24 °C

Post-larval development ended at day 7. Stolon growth was more rapid than at 18 °C, with three functional gastrozooids at day 9 and six at day 10. Eudoxids were produced between days 10 and 11, but after day 14, all specimens of the first generation degenerated. In this experiment, 76 specimens grew to be young colonies and they liberated 186 eudoxids.

(*c*) *Generation time*

Three phases in the generation time of diphyid siphonophores can be distinguished (figure 3). The first corresponds to larval and post-larval development; the second, or polygastric phase, to the growth of the young colony up to the liberation of the first eudoxid; and the third relates to the growth of the eudoxid until male or female gametes are released from the first gonophore to reach maturity.

At 18 °C, the larval and polygastric phases last 12–14 days, and at 24 °C, 10–11 days. Eudoxids liberated at the two temperatures were cultured in order to determine the length of the third phase in each case. At 18 °C, the eudoxids liberated between day 12 and 14 produced a mature gonophore-releasing gametes from day 19, whereas at 24 °C, eudoxids liberated between days 10 and 11 began to release gametes from day 15. Thus generation time (egg to egg) for *M. kochi* is 19 days at 18 °C, and 15 days at 24 °C. At 13 °C, it was not possible to estimate generation time, as we did not obtain liberation of eudoxids. However, after three weeks at this temperature, the colonies had reached a size at which they were capable of liberating eudoxids.

(*d*) *Life span of eudoxids*

When liberated, each eudoxid is made up of a bract, a gastrozooid with its tentacle and a gonophore (figure 1 *i*). The gonophore is at the same time the locomotor organ of the eudoxid, and the reproductive organ (the gametes are differentiated from its manubrium). After releasing the gametes, the gonophore detaches from the remainder of the eudoxid and degenerates. It is replaced by a secondary gonophore, already partially developed before the loss of the preceding gonophore. Each gonophore may be either male or female, and the same eudoxid may develop successively male or female gonophores, with an irregular alternation of the two. In culture, we have followed the same eudoxid producing over four successive generations of gonophores.

4. DISCUSSION

Muggiaea kochi was chosen for the study because it is a very abundant species that has an important impact

on the Mediterranean planktonic ecosystem. We have been able to obtain, for the first time in the laboratory, a complete life cycle of a siphonophore from egg to egg. As might be expected from the small size of the adult colonies and the rapid appearance of blooms of *M. kochi* in the late spring, the life-cycle is short at temperatures $\geqslant$ 18 °C and strictly a function of temperature: 19 days at 18 °C, 15 days at 24 °C. On the contrary, in our cultures at 13 °C, the polygastric phase was stationary.

In the laboratory, development is accelerated at 24 °C and at the same time, more eudoxids are produced compared with the cultures at 18 °C, the temperature at which the species is most abundant in the sea. This difference between the optimal conditions in the laboratory and in the sea presumably is due to the fact that parameters other than ambient temperature may affect the life cycle of *M. kochi* in the sea; for instance, trophic factors (Purcell 1982) which are variable in the sea and constant under laboratory conditions whatever the temperature.

Both at 18 °C and at 24 °C, the period of eudoxids release was short: three days at 18 °C and two days at 24 °C (figure 3), and always was followed by the death of first-generation specimens. In contrast, at 13 °C, at which temperature no eudoxids were liberated in our cultures, 26 specimens of the first generation still survived and showed no sign of degeneration for up to 21 days, after which time the experiment was stopped. Siphonophores are very difficult animals to maintain in culture, so that the presence of 26% of the specimens after three weeks suggests that in the sea at 13 °C (the temperature of the Mediterranean in February and March), the polygastric stages could exist for several weeks without eudoxid production. Thus these results lead us to propose that for this species there are two types of cycle, differing in duration rather than in kind.

1. A short spring cycle of 2–3 weeks at 18 °C which occasionally is repeated in the autumn. There are three phases: larval development, growth of the polygastric colony with the development of several immature eudoxids and growth and maturation of the eudoxids and the release of several gonophores.

2. A long winter cycle at 13 °C, differing from the spring cycle in that the second phase is a long stationary polygastric phase which, in all likelihood, begins to produce the third or eudoxid phase only at the warming of the sea in spring.

Our observations over three decades, on the temporal distribution of *M. kochi* in the Bay of Villefranche-sur-mer, have shown that this is a neritic species that blooms in spring (2–3 nectophores per cubic metre in May and June 1984), and sometimes in Autumn (0.3 nectophores per cubic metre in October 1984), as both polygastric and eudoxid stages. Outside these periods, a small number of polygastric colonies and eudoxids usually is present in the surface water (0–50 m) except in certain years when, during short (1–3 weeks) periods in February or March, only polygastric stages are present.

Comparison of the results obtained from cultures and from *in situ* observations suggests that the short and long cycles seen in the cultures also exist *in situ*. Nevertheless between these two ends of the spectrum, cycles of intermediate duration with reduced eudoxid production must exist at intermediate temperatures. It should be mentioned that we have never identified the existence (*in vitro*) of the benthic phase suggested by Rottini (1974) during the life cycle of *M. kochi*. In our opinion, all siphonophores are holoplanktonic with the exceptions of *Physalia physalis*, the well-known pleustonic cystonect, and the physonect family Rhodaliidae, little known until Pugh's (1983) observations definitely demonstrated their benthic mode of life.

In the Ligurian Sea, during the two decades up to 1981 only the single *Muggiaea* species, *M. kochi*, was known to be abundant in the spring. *M. atlantica* occurred rarely and only for short periods at the same time as *M. kochi* was present. Simultaneous blooms of the two species were never observed, and the two were considered to be mutually exclusive (Russell 1934; Mackie *et al.* 1987). After 1982, there was an abundant population of *M. atlantica*, together with the continuance of an abundant population of *M. kochi*. Since 1987, *M. atlantica* has predominated, with *M. kochi* becoming the minor species present (C. Carré & D. Carré, unpublished observations). Gill *et al.* (1987) have noted a similar change in the neighbouring Catalan sea.

The study on *M. kochi*, which will be extended eventually by a similar study on *M. atlantica*, should allow a better understanding of the recent establishment of *M. atlantica* in the Ligurian Sea, and more generally, the fluctuations over the years of the two species (long-term ecological studies), doubtless related to long-term hydrological and meterological cycles.

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