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THE LIFE CYCLE OF PLATYMONAS IMPELLUCIDA MCLACHLAN ET PARKE (PRASINOPHYCEAE) IN CULTURE*

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An asexual life cycle of *Platymonas impellucida* was established in culture. Cells incubated on a light:dark cycle of 18:6 h settled from the medium and attached to the culture vessel 2 hours before initiation of the dark period. Cells divided in darkness, and daughter cells were released from the parent theca shortly thereafter. Thick-walled cysts were formed in abundance after 2 and 8 weeks of incubation in enriched or synthetic seawater respectively. Differences between media of enriched or synthetic seawater with respect to formation of cysts were not resolved. However, attempts to accelerate formation of cysts in the synthetic medium indicated that nutrient deficiency, variation in pH, and slow desiccation had effects. Tolerance of cysts to extremes of temperature and desiccation was no greater than that of motile cells.

Introduction

Life cycles in the Prasinophyceae have received little attention, and, with the exception of one unconfirmed report of sexuality (Gorbunova 1961), only asexual reproduction is known in this class. The life cycle of species of *Platymonas* has been investigated from several aspects. Stewart and Mattox (1975) and Stewart et al. (1974) examined characteristics of cell division and nuclear phases of this genus. Morphological changes in the life history, including formation of cysts and subsequent germination, have been emphasized. Recently the life cycle of an unnamed species of *Platymonas* has been completed (Tanoue & Aruga 1975; Kobara & Hori 1975), with Grant and Vadas (1976) providing additional information for *P. subcordiformis*.

In the present instance I show that the asexual cycle of *P. impellucida* is similar to that described previously for *Platymonas* sp (Tanoue & Aruga 1975), but with certain morphological differences. I have also examined factors leading to formation of cysts, and tolerances of these cysts to temperature and desiccation.

Materials and Methods

Platymonas impellucida McLachlan et Parke was isolated from a sample of water from Puerto Rico (McLachlan & Parke 1967), and this species is No. 161/5 in the Cambridge Culture Collection; cultures are also maintained at the Atlantic Regional Laboratory, Halifax, and at the Marine Biological Laboratory in Plymouth.

An axenic culture was maintained in a synthetic medium, ASP-M, and experimental work was done using this and an enriched seawater medium, SWM-1 (McLachlan 1973). Incubation was in 30 ml of medium contained in 125 ml erlenmeyer flasks capped with shortened glass beakers, the flasks being agitated daily. Cultures were maintained at 25°C; illumination was provided by 40-W, cool-white fluorescent lamps at an intensity of 100μE m^{-2} s^{-1} at the level of the flasks, and the light:dark cycle was 18:6 h.

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Media deficient in nutrients were established by omitting individual compounds from ASP-M. Cells growing exponentially were sedimented by centrifugation and resuspended in medium lacking the indicated compound; the initial concentration of cells was adjusted by dilution to 2×10^4 cells ml^{-1}. Microscopic examination of the cells was made after 3 weeks of incubation.

Medium deficient in carbon was effected by autoclaving ASP-M lacking added bicarbonate in 125-ml screw-capped erlenmeyer flasks; following autoclaving and cooling, the caps were cinched, thus limiting gaseous diffusion into the flask (McLachlan & Craigie 1966); controls were established by the addition of filter-sterilized bicarbonate to each flask, bringing the concentration to 10 mM $NaHCO_3$.

Salinity in the synthetic medium was varied by varying the concentrations of NaCl, $MgSO_4$, $MgCl_2$, $CaCl_2$, and KCl while maintaining a constant ratio amongst these salts. Normal salinity of the medium is designated as "X". Other additives were provided at the same concentration independent of salinity.

Effects of pH on formation of cysts were determined in ASP-M containing 10 mM of either glycylglycine or tris buffers to minimize changes in pH. This is twice the normal concentration of buffer, and no effect on rate of growth or final yield of cells resulted from these increases. Cells for inoculation were collected by centrifugation and resuspended into medium of the experimental pH, the initial density of cells being adjusted to 5×10^4 cells ml^{-1} Concentration of cysts was determined, using a haemocytometer, after 3 weeks of incubation.

Effects of pH on formation of cysts were examined also using membrane filters and membrane-filter pads. Cells for inoculation were centrifuged and resuspended in ASP-M containing 10 mM glycylglycine at the experimental pH; 1 ml of this suspension was gently filtered onto membrane filters (0.45 μm pore size), and these filters were placed into glass petri dishes of 60 mm diameter, some containing discs of filter paper (Whatman No. 1) saturated with the experimental medium. Both procedures were tested with the concentration of cells ranging from 2×10^4 to 5×10^6 cells ml^{-1}, and cells from early exponential, exponential, stationary, and late stationary stages of growth (i.e. 2, 5, 10, and 14 day-old cells respectively). Incubation was in the light under standard conditions, and membrane filters alone became dehydrated within 24 hours and those on filter pads within 3 days; when dry, membranes were examined for viable cells and cysts.

Resistance of motile cells and cysts to temperature and desiccation involved cells grown in SWM-1, 1/10 SWM-1, ASP-M, and 1/10 ASP-M; 1/10 media contained additives of SWM-1 at 0.1 of the specified concentration. Cells from a stage of growth, ranging from 2- to 28- day-old cultures, were collected on membrane filters (0.45 μm) at a concentration of 10^6 cells, and these filters were placed on pads of filter paper saturated with the appropriate medium in glass petri dishes. Two collections, each in triplicate, were made from each age of cells; one group was placed at -2°, 40°, 80°C or in a desiccator with silica gel, and the second group was returned to normal conditions of incubation; after 3 days, microscopic examination was made for the presence of cysts. When cysts were present these filters were transferred to -2°, 40°, 80°, and in a desiccator with silica gel. After 1 hour at the different temperatures or 1 week in the desiccator, filters were removed and placed in tubes containing SWM-1 and incubated under normal conditions; evidence of survival (i.e. presence of motile cells) was determined 2 and 4 weeks after inoculation.

Results

Life Cycle

A light:dark cycle of 18:6 h synchronized cellular division, allowing monitoring of cells during exponential growth. Cells remained motile and single during most of the light period, and after about 16 hours they attached apically to the bottom and sides of the vessel. Once attachment occurred, flagella were discarded. Cell division oc-

curred during darkness resulting in 2 motile daughter cells, and release from the parental theca was during darkness or early in the light period. The actively motile daughter cells mechanically ruptured the parental theca and escaped, and these cells were positively phototactic, gathering at the surface of the medium.

There were always a few cysts in a culture, the percentage being less than 1% during exponential growth, increasing as the culture entered the stationary phase of growth and the cells became older. In stationary phase there was a combination of vegetative cells (single, motile cells, and thecae containing 2 daughter cells) and cells in cyst phase (thecae containing 4 daughter cells, non-motile, spherical cells, and thick-walled cysts).

Cyst formation involved accumulation of starch granules by motile cells, resulting in a more spherical shape. These cells then attached apically, discarded the flagella and formed a thick cell wall. Such cells are referred to as 'immature' cysts, and those which formed short spines protruding randomly from the cell wall are referred to as 'mature' cysts. Both types of cysts germinated in an identical manner. Transfer of cysts to fresh medium resulted in a loss of the granular appearance, followed by division into 4 motile daughter cells. Daughter cells, enclosed in a parental theca, were released from the cyst by a rupture in the wall. Escape from the theca was as described before, there being no difference between these cells and those produced in binary division.

Cyst Formation

In SWM-1 the stationary phase of growth resulted after 7 days of incubation, and during the next 7 days, mature cysts formed with thick walls and short spines. In ASP-M, only a few cells became spherical during the first week of the stationary phase. Although most cells accumulated some starch, these cells never became spherical until at least 6 weeks after inoculation; at this point they had thickened walls devoid of spines.

Acceleration of the formation of cysts was attempted by rendering ASP-M deficient in various components, and these results are summarized in Table 1. Formation of cysts occurred in none of these treatments after 3 weeks of incubation. Although several treatments resulted only in minimal growth, final yield of cells in most being similar to that of the control.

At salinities ranging from 0.1 to 3 times that of the normal salinity of ASP-M, a period of 6 to 8 weeks was required before cysts began to form, although no growth occurred in 0.1 salinity. In all other treatments, the cyst to cell ratio was similar, the concentration depending upon the salinity, and almost all cysts were immature. Formation of mature cysts, therefore, was not related to salinity.

In a range of pH values from 7.0 to 9.5, using both glycylglycine and tris buffers in ASP-M, cysts were present at 1% or less at all pH's after 3 weeks of incubation.

Following dehydration on membrane filters over 24 hours, no viable cells remained, and none had formed cysts. Contrariwise, large numbers of cysts formed on membrane filters on pads of filter paper (Table II); these cysts were immature, lacking spines. This technique resulted in high concentrations of cysts, although it was not always reproducible, and frequently the cells lysed. However, in trials where cysts were formed, percentages were comparable to those in Table II. Concentration of cells and age of cells were varied independently in an attempt to obtain cysts consistently, but none of these combinations was successful.

Cells inoculated into closed flasks containing ASP-M with added 10 mM $NaHCO_3$ produced after a week of incubation a final yield of cells comparable to that in open flasks. Following a further period of 7 weeks, cells in the open flasks had produced thick-walled cysts, whereas cells in the closed flask were non-viable and had bleached although flagella remained attached to these cells; cysts or cells filled with starch particles were absent.

After 6 weeks on slants of agar (1.5% agar in SWM-1) at 25°C, 25μE m^{-2} s^{-1}, both

Table I. Cell conditions as a function of nutrient deficient media; examined after 3 week in medium lacking indicated compound

Deficient compound	% Cysts	Motile	Attached to flask	Growth	Remarks
$NaNO_3$	0	-	-	-	Spherical cells with large conc. starch
NaH_2PO_4	0	-	-	-	Spherical cells
Na_2EDTA	0	+	+	+	
$FeCl_3$	1	+	+	+	
P-I metals	0	+	+	+	
TMS-II metals	0	+	+	+	
Vitamins	0	+	+	+	
$MgSO_4$	0	+	+	+	Deformed chloroplasts
$MgCl_2$	0	+	+	+	
$CaCl_2$	0	-	-	-	Non-motile with flagella present
Control	0	+	+	+	

Table II. Cyst formation as a function of pH and desiccation

	% Cysts	
pH	Membrane Filter	Membrane filter + filter pad
6.0	-	10
7.0	-	40
7.5	-	95
8.0	-	5
9.0	-	40
10.0	-	40

dead and viable cells were noted. The latter consisted of normal motile, single cells together with non-motile, clumped cells which were spherical and enclosed in at least 2 thecae. Thick-walled cysts were not observed, even after 18 months on agar, but some cells remained viable, and noticeable growth occurred within 4 days when transferred to fresh liquid medium.

Transfer of cells in exponential growth at 25°C to other temperatures did not stimulate formation of cysts. Cells in exponential growth at 20°C were more spherical than those at 25°C, and with an apparently greater number of starch bodies. At 35° or 40°C, cells were spherical, filled with starch, and non-motile. Each theca contained up to 20 spherical cells of different sizes. The diameter of these thecae ranged from 40 to 60 μm, and intrathecal spores ranged in size from 5 to 15 μm which was smaller than the 18 μm average diameter of non-motile cells. Transfer of these cells to 25°C resulted in motile single, flattened cells. Not all spherical cells in enlarged thecae, however, were viable as evidenced by an increased amount of

cellular debris in the medium. Changes in morphology of cells when transferred from 25° to 35°C and then returned to 25°C are illustrated in Figure 1.

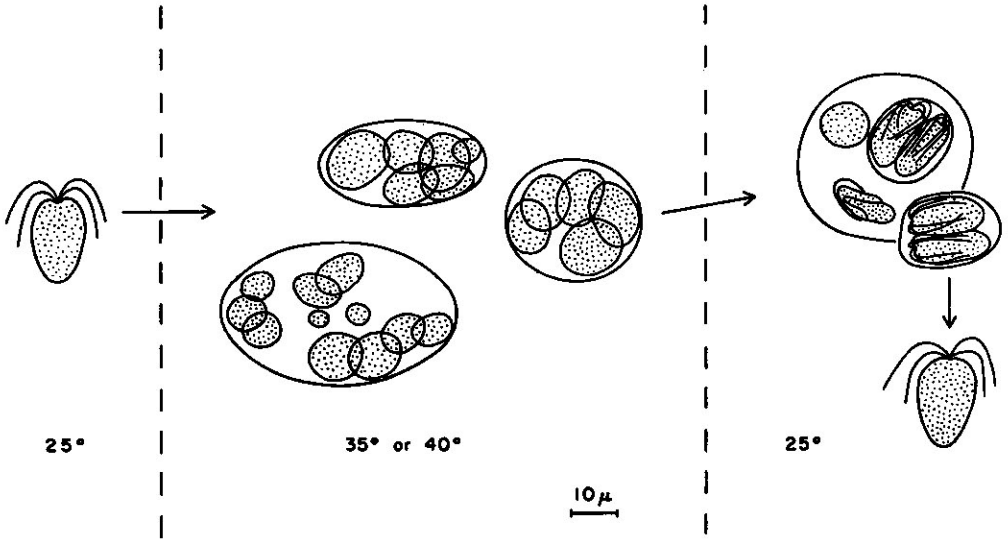

Fig 1. Life cycle of *Platymonas impellucida*

Temperature and Tolerance to Desiccation

The ability of cysts and cells to survive extremes of temperature and desiccation is summarized in Table III. Cells in early exponential growth survived -2°C and 40°C regardless of the medium. Cells from early stationary phase (7 d) and late stationary phase (28 d) of growth were susceptible to all temperatures.

Neither cysts nor cells survived 7 days of desiccation, and in general cysts were no more tolerant of extremes than motile cells.

Discussion

The life cycle of *Platymonas impellucida* resembles that of *Platymonas* sp. (Tanoue & Aruga 1975), but there are several noticeable differences between these 2 cycles. The most obvious being that the 2 daughter cells of *Platymonas* sp were non-motile and attached when they left the parental theca. Further, release from the parent theca was independent of light, although separation of cells was stimulated by light. In contrast, daughter cells of *P. impellucida* formed flagella before separation, and on separation, these cells were actively motile, although retained within the parental theca. The daughter cells swam randomly until rupture of the parental theca resulted. Cellular division in *P. impellucida* usually occurred during darkness with release from the parental theca shortly thereafter, also usually in darkness although some cells were released in the early portion of the light cycle. Other species of *Platymonas* behave similarly to *P. impellucida*. *Platymonas subcordiformis* relies on motile daughter cells to rupture the parental theca (Grant & Vadas 1976) and not the enzymatic method suggested by Tanoue and Aruga (1975) for their species.

Duration in the double-celled condition usually was 2 to 4 hours for *P. impellucida*, and within 2 hours after initiation of the light cycle, all cells were single and motile. Similar observations have been made on *P. tetrathele* (Gooday 1970) and

Table III. Tolerance of motile cells and cysts to temperature (−2°, 40°, 80°C) and desiccation (D) as a function of cell age and medium

Cell age (days)	Medium	Motile cells −2°	40°	80°	D	Induced cysts −2°	40°	80°	D
2	SWM-1	−	+	−	−	+	−	−	−
	1/10 SWM-1	+	+	−	−	−	−	−	−
	ASP-M	+	+	−	−	−	−	−	−
	1/10 ASP-M	+	+	−	−	+	−	−	−
7	SWM-1	−	−	−	−	−	−	−	−
	1/10 SWM-1	−	−	−	−	−	−	−	−
	ASP-M	−	−	−	−	−	−	−	−
	1/10 ASP-M	−	−	−	−	−	−	−	−
14	SWM-1	+	−	−	−	+	−	−	−
	1/10 SWM-1	−	−	−	−	−	−	−	−
	ASP-M	−	−	−	−	−	−	−	−
	1/10 ASP-M	−	−	−	−	−	−	−	−
21	SWM-1	+	−	−	−	−	−	−	−
	1/10 SWM-1	−	−	−	−	−	−	−	−
	ASP-M	−	−	−	−	−	−	−	−
	1/10 ASP-M	−	−	−	−	−	−	−	−
28	SWM-1	−	−	−	−	−	−	−	−
	1/10 SWM-1	−	−	−	−	−	−	−	−
	ASP-M	−	−	−	−	−	−	−	−
	1/10 ASP-M	−	−	−	−	−	−	−	−

other species of *Platymonas* (Tanoue & Aruga 1975). In all of these species the duration as double cells generally was 2 to 4 hours. However, one species, *P. convolutae,* studied by Gooday (1970), spent most of the light period (20 h) in the double-celled condition, a behavior common to species of *Prasinocladus.*

The fate of flagella differs amongst species. *Platymonas impellucida* behaves as other species of *Platymonas* and *Prasinocladus* studied by Kobara and Hori (1975). Once firmly attached, flagella were discarded and were apparent in the medium. In other species, such as *Platymonas* sp (Tanoue & Aruga 1975), the flagella remain at-

tached to the parental theca even after release of daughter cells. Parental cells of *P. subcordiformis* (Grant & Vadas 1976) released their flagella before attachment. Thus attachment at any point on the parental theca is indicated and not only at the apex, the point where most species of *Platymonas* attach.

The mechanism of attachment is not fully understood. Manton and Parke (1965) reported that species of *Platymonas* and *Prasinocladus* have flagella covered with a thick layer of mucus containing scales. Initial attachment may be a function of this mucilage, holding the cell to the substrate and allowing permanent attachment to occur. This suggestion is not applicable to *P. subcordiformis* which discards its flagella before attachment. However, it has been suggested that *P. subcordiformis* excretes an extracellular adhesive which attaches the parental cell to the substrate, and no metabolic energy is required to maintain this attachment (Grant & Vadas 1976).

The life cycle of many species of Prasinophyceae includes formation of thick-walled, spherical cysts. The production of cysts, most possessing short spines protruding from the surface, is a generic characteristic of species of *Platymonas* (Parke & Manton 1967). Tanoue and Aruga (1975) and Kobara and Hori (1975) have reported on formation of cysts and subsequent germination. Cysts of *Prasinocladus* are the same as for species of *Platymonas* (Kobara & Hori (1975). *Platymonas impellucida* follows the same pattern, with each cyst producing 4 motile daughter cells.

Although formation of cysts apparently is an integral part of the life history of the Prasinophyceae, conditions for formation of cysts are poorly understood. Generally cysts are considered to form under 'unfavorable conditions', although such conditions have not been specified (Peterfi & Manton 1968; Hori & Chihara 1974).

Platymonas impellucida invariably formed a few cysts, even during exponential growth, and after 4 weeks of incubation in ASP-M, about 1% of the cells were as cysts. A large proportion of the cells were as cysts after 6 to 8 weeks, a similar period of time being required by Tanoue and Aruga (1975) to induce cysts in *Platymonas* sp incubated in a synthetic medium. If the medium became deficient in carbon, *P. impellucida* showed no tendency to formation of cysts (i.e. rounding of cells and accumulation of starch), even after 8 weeks. Contrariwise, *P. impellucida* formed large numbers of cysts within 2 weeks if incubation was in SWM-1. Differences between these 2 media, with respect to formation of cysts, were never resolved. There was some indication that the iron component of ASP-M was not entirely adequate for all cellular functions, and reduced chlorophyll a/cell was obtained in this medium. However, rates of growth and final yields of cells were nearly identical for each medium (unpubl. results).

Slow desiccation accompanied with variation in pH stimulated formation of cysts. Minimal numbers of cysts formed around pH 8, the normal pH of seawater (Harvey 1957). Formation of cysts in *P. impellucida* and other species (Tanoue & Aruga 1975) occurred with variation in hydrogen ion concentration and *P. impellucida* also formed cysts at pH values which did not support growth (i.e. pH 9.5 and 10.0) (unpubl. results), suggesting that cysts function as a protective mechanism. Modification of pH without desiccation or modification of pH with rapid desiccation failed to induce formation of cysts and in the latter case, no cells survived.

Low tolerance of extremes in temperature and long periods of desiccation casts doubt on the ecological significance of cysts. Although in formation of cysts, cells underwent a 3-day period of desiccation, they were less tolerant of extremes in temperature than were motile cells. None of the cells or cysts withstood 7 days of desiccation over silica gel. The observation that cysts are not more resistant to extremes of temperature and desiccation than motile cells is not unusual. Evans (1958; 1959) suggested that in freshwaters, the ability to survive desiccation and heat was not related to the ability to form cysts. Belcher (1970) investigated effects of temperature and desiccation in 11 species of freshwater Prasinophyceae, and was able to show increased tolerance of cysts over the motile cells in only 7 species.

Considering cysts of *P. impellucida* as a protective mechanism has some appeal. General depletion of nutrients, variation of pH from the norm, and increase in salinity, all induced formation of cysts. Increases in temperature, which induced formation of cysts in other species of *Platymonas* and *Prasinocladus* (Kobara & Hori 1975), did not stimulate formation of cysts in *P. impellucida*. Whether cysts of species of marine Prasinophyceae are more resistant to drought and temperature than motile cells requires additional study. However, conditions for formation of cysts in *P. impellucida* indicate that cysts may have ecological advantage.

References

Belcher, J.H. 1970. The resistance to desiccation and heat of the asexual cysts of some freshwater Prasinophyceae. *Br. Phycol. Bull.* 5:173-77.

Evans, J.H. 1958. The survival of freshwater algae during dry periods. Part I. An investigation of the algae of five small ponds. *J. Ecol.* 46:149-67.

Evans, J.H. 1959. The survival of freshwater algae during dry periods. Part II. Drying experiments. Part III. Stratification of algae in pond margin litter and mud. *J. Ecol.* 47:55-81.

Gooday, G.W. 1970. A physiological comparison of the symbiotic alga *Platymonas convolutae* and its free-living relatives. *J. Mar. Biol. Assoc., U.K.* 50:199-208.

Gorbunova, N.N. 1961. Polovio protsess u *Asteromonas gracilis* Artari (The sexual process in *Asteromonas gracilis* Artari). Bot Zh. Kyyiv. 46:993-98.

Grant, W.S. and **Vadas, R.L.** 1976. A diurnal settling rhythm in *Platymonas subcordiformis* Hazen. *J. Protozool.* 23:557-59.

Harvey, H.W. 1957. *The Chemistry and Fertility of Sea Water.* Cambridge Univ. Press, New York.

Hori, T. and **Chihara, M.** 1974. Studies on the fine structure of *Prasinocladus ascus* (Prasinophyceae). *Phycologia* 13:307-15.

Kobara, T. and **Hori. T.** 1975. On the formation and germination of cyst in *Platymonas* sp and *Prasinocladus* spp (Prasinophyceae). *Bull. Jpn. Soc. Phycol.* 23:111-15.

Manton, I. and **Parke, M.** 1965. Observations on the fine structure of two species of *Platymonas* with special reference to flagella scales and the mode of origin of the theca. *J. Mar. Biol. Assoc. U.K.* 45:743-54.

McLachlan, J. 1973. Growth media-marine. In: *Handbook of Phycological Methods.* (ed. J. Stein). Cambridge Univ. Press, London. pp. 25-51.

McLachlan, J. and **Craigie, J.S.** 1966. Chitan fibres in *Cyclotella cryptica* and growth of *C. cryptica* and *Thalassiosira fluviatilis.* In: *Some Contemporary Studies in Marine Science.* (ed. H. Barnes). George Allen and Unwin Ltd., London. pp. 511-17.

McLachlan, J. and **Parke, M.** 1967. *Platymonas impellucida* sp. nov. from Puerto Rico. *J. Mar. Biol. Assoc. U.K.* 47:723-33.

Parke, M. and **Manton, I.** 1967. The specific identity of the algal symbiont in *Convoluta roscoffensis. J. Mar. Biol. Assoc. U.K.* 47:445-64.

Peterfi, L.S. and **Manton, I.** 1968. Observations with the electron microscope on *Asteromonas gracilis* Artari emend. [*Stephanoptera gracilis* (Artari) Wisl.], with some comparative observations on *Dunaliella* sp. *Br. Phycol. Bull.* 3:423-40.

Stewart, K.D. and **Mattox, K.R.** 1975. Comparative cytology, evolution and classification of the green algae with some consideration of the origin of other organisms with chlorophyll *a* and *b*. *Bot. Rev.* 41:104-35.

Stewart, K.D., Mattox, K.D., and **Chandler, C.D.** 1974. Mitosis and cytokinesis in *Platymonas subcordiformis,* a scaly green monad. *J. Phycol.* 10:65-79.

Tanoue, E. and **Aruga, Y.** 1975. Studies on the life cycle and growth of *Platymonas* sp. in culture. *Jpn. J. Bot.* 20: 439-60.