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John Dindo
Robert MacGregor
George Crozier

University of Alabama in Birmingham

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Department of Biology
University of Alabama in Birmingham
Birmingham, Alabama

Dauphin Island Sea Lab
Dauphin Island, Alabama

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JOHN DINDO, ROBERT MACGREGOR AND GEORGE CROZIER
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Analysis of Reproductive Hormones and Plasma Lipid Levels Associated with the Migration of the Stripped Mullet, Mugil cephalus L.

JOHN DINDO, ROBERT MACGREGOR AND GEORGE CROZIER
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Mugil cephalus, a ubiquitous estaurine fish, makes an annual migration to the open sea to spawn. The specimens for this project were caught within the lower and middle reaches of Mobile Bay. Monthly samples of the reproductive steroids (testosterone and estrogens) were analyzed for a one year cycle utilizing a radioimmunoassy technique. Plasma lipid levels were analyzed by use of standard clinical methods utilizing spectrophotometry. Initial analysis shows that male plasma testosterone levels begin a rapid rise from April through August. Females showed a slight rise in plasma estrogens from March to May followed by a decline in July. The gonadal somatic index at this time does not reflect early steroid production. The plasma lipids, total lipids and triglycerides, show a rise from April with a peak being attained in June for both males and females. Cholesterol, however, shows a decrease in level from April to June for both males and females. A peak in reproductive steroids is predicted for November with spawning to occur at the end of December and early January.

Annual Cycle Of Gonadal Growth

This study presents the related reproductive changes during an annual cycle of testes growth and serum androgens in male, and ovarian growth and serum estrogens in females, along with several serum lipids from both male and female striped mullet, Mugil cephalus.

The striped mullet lives most of its life in the highly complex estuarine habitat. This fish annually undergoes an explosive development during which reproductive steroids and gonadal mass of the animals increase approximately four fold in a three month period. Striped mullet migrate offshore to spawn (Anderson, 1957; Futch, 1966; Moore, 1974). This study indicates that spent adults are returning to the estuaries in December. The times of migration and spawning in this study agree with other studies on the striped mullet (Broadhead and Mefford, 1954; Abraham et al, 1966). In a recent study (Finnucane, 1979) striped mullet eggs were found 50-60 miles offshore on the continental shelf in the Gulf of Mexico. This indicates a Ocotber-November spawn as does the material presented in this study. The salmonids undergo similar inland migrations to spawn (Idler et al, 1954, 1959, and 1960). Numerous studies on these animals and other fall spawners have shown that short days and changing temperatures are keys to stimulation of reproductive behavior (Baggerman,

1969, 1972; Kuo et al, 1974; MacQuarrie et al, 1978; and Whitehead et al, 1978). It has been observed in this study that when the natural photoperiod is shortening (less than 12 hours) and when the temperature falls to approximately 20°C in September and October, concurrently with the initiation of rapid gonad growth and reproductive readiness. The relationships of shorter photoperiod and changing temperatures affecting reproductive cycles in fish has been noted by Reinboth (1972).

The Gonado-Somatic Index and the gonad weights both exhibit a distinct rise in the month of October. Both are reflections of the change in gonadal mass occuring at this time. Body lengths and weights are used in this study as an index of sexual maturity (Kuo et al, 1973, personal communication with Kuo, 1979). This study has examined the possibility of different age classes appearing in spring and summer samples versus samples caught in the reproductive season. The expression of gonad weights as a percentage of body weight does not significantly change the picture, although a wide range of adult weights were observed in the samples. All animals used in this study were considered to be reproductive adults based on total body length within 23-37 cm (Jacot, 1920; Futch, 1966; Miller, 1971; Kuo, 1973, 1979). Broadhead (1953) and Thompson (1963) reported schooling segregation by size. Table 2 shows a significant change for both males and females for body length ($P < .001$) by month and by sex. Throughout the study period females

were significantly longer and heavier than the males. Other researchers have observed similar size distinction in their studies (Jacot, 1920; Idyll and Sutton, 1951; Miller, 1971; Moore, 1974; and Major, 1978).

Males and females during premigratory months (March-September) have lengths averaging 27.71 cm and 30.82 cm respectively. The fall fish show a 30% increase in length to 36-40 cm for males and females. Most researchers believe that striped mullet attain reproductive capacity in their second year, attaining a length of 23-37 cm(Jacot, 1920; Broadhead, 1956; Futch, 1966; and Miller, 1971). Anderson (1954) indicated in his study on the striped mullet that the average size of spawning fish in southern waters was 18.9 cm for males and 20.9 cm for females. The increase in size of approximately 10 cm from spring to fall can not be explained by summer growth, more likely it reflects the size bias created when sampling migratory schools.

Serum Gonadal Steroids

The onset of serum steroid increase for both sexes occurs in October peaking in November (Fig. 7, and Tables 3 & 4), which coincides with the gonadal cycle. These patterns of serum steroids are similar to the Pacific salmon (Schmidt and Idler, 1962). In sockeye salmon, Onchornchus nerka, reproductive steroid levels rose late in the phases of spawning (Schmidt and Idler, 1962). Abraham

et al (1966) found similar changes in Mugil cephalus and Mugil capito. Wingfield and Grimm (1976) suggest that testosterone may act as a precursor for estrogen synthesis in females during the vitellogenic season. The positive correlation seen in this study between steroid levels and Gonado-Somatic Index has been expressed in other studies (Woodhead, 1975; Grimm and Evans, 1978; Lambert et al, 1978; and Wingfield and Grimm, 1978). Both serum steroid levels and GSI may be used as an index of gonadal condition throughout the year. Examples of this are seen in the estradiol levels (in females) and GSI changes in the northern plaice Pleuronectes platessa and in the rainbow trout, Salmo gairdneri (Wingfield et al, 1976; and Crim and Idler, 1978). A rise in estrogens in some fish has been noted to stimulate vitellogenesis (Woodhead, 1975; Crim and Idler, 1978; and Lambert et al, 1978). The most prevalent form of estrogens in marine fish are estradiol and estrone (Hoar, 1969). The present antiserum had strong cross reaction with estradiol 17β (100%), - 17β (25%) and estrone (50%) with only a 5% cross reaction with estriol.

The androgens in male striped mullet follow the same pattern of rapid increase initiating in October as do the estrogens in the females. The testosterone levels are maintained in a slightly lower concentration than estrogens throughout the prespawn months but rise twice as high as the estrogens during the spawning period. In males, several

serum androgens have been identified; testosterone, 11-ketotestosterone (in sockeye salmon, Oncorhyncus nerka, and the Atlantic salmon Salmo salar, and the northern plaice Pleuronectes platessa (Idler et al, 1960; Idler et al, 1964; Eckstein and Eylath, 1970; and Schrek, 1972).

Serum Lipids

The annual migrations of striped mullet to offshore waters in the fall is dependent on key metabolic changes which enable these animals to mobilize energy reserves. Allen (1976) stated that it was unquestionable that the function of lipids as energy reserves of high free energy content per unit weight is one of the most dynamic functions of lipid compounds. Hoar (1960), Woodhead (1975), and Perera et al, (1978) indicate that lipids play a major role in providing simultaneous metabolic energy for migration and gonadal development in young mullet. Seasonal variation in serum total lipids and cholesterol (Fig 8) indicates a period of rapid deposition of lipids during the summer months. This coincides with large plankton blooms and large growth of the submerged sea grass, Rupia maritima (Odum, 1968). Broadhead (1956) and Anderson (1958) have noted heavy premigratory feeding in the striped mullet. Present observations have indicated extensive mesenteric fat throughout the summer months, and has been observed by other researchers (Hoar, 1960; Woodhead, 1975). The fall in serum total lipids and cholesterol coincide with the time of

rapid lipid deposition (Deng et al, 1976; Perera and DeSilva 1978). Many migration fish meet their energy demands by feeding during the migration. Dietary intake of lipids and synthesis from non-lipoidal substances (carbohydrates) supplies the animal with large energy reserves (Benson and Lee, 1975). The striped mullet, similar to the Pacific salmon, do not feed during the migration to offshore spawning areas (Odum, 1968). Salmon accumulate large stores of fat before the start of their migration. Greene (1926) could detect very little stored lipid in the bodies of salmon found on the spawning grounds.

The serum total lipids for both male and female striped mullet follow a significant seasonal pattern (Table 6, Fig. 8). In October the sharp increase in serum total lipids is maintained through the spawning months. The increase in cholesterol in October and November may provide steroid precursors (Bentley, 1976). However, the main increase in the serum lipids and cholesterols is associated with transport functions of lipids and cholesterols (Idler and Schmidt, 1959). In December the serum total lipids and cholesterol decrease in the utilization coincident with post migratory periods. Perera and DeSilva (1976) found similar seasonal patterns in total serum lipids of mullet caught in southeast Florida region. Their lowest values were obtained from January to July with the highest levels being found in September and November. Deng et al (1976)

found that body lipids followed the same type pattern as this study has observed for the serum total lipids. Body lipids ranged below 15% of the animals wet weight from January to July but rapidly increased in the early fall to above 30% in October.

Serum triglycerides showed a very erratic fluctuations throughout the monitoring period, possibly reflecting the diet of these animals. Triglycerides and wax esters are main types of neutral lipids for storage in most animals but in marine animals they do not represent the major lipid reserve (Barnes and Blackstone, 1973: Malins and Sargent, 1976).

Greene (1926) estimated that the majority of stored lipid in salmon was utilized for gonadal development. Channum and ElSaby (1952) found that the herring had 15% of its body weight as fat which was utilized for gonad growth during its migration. Because striped mullet do not feed during their migration and spawning (Honmans and Vladikov, 1954; Thompson, 1954; and Odum, 1968), they must rely on fat reserves for energy needs. It is felt that rapid rises in serum lipids may reflect mobilization of stored fats for the onset of spawning. Greene (1926) showed a spectacular growth of the ovaries during a non-feeding migration in Salmo salar, the steelhead trout. Similar types of growth rates of the gonads in striped mullet have been observed by Miller (1971) and Moore (1974). Early in Oct-

ober, dissection of these animals revealed the intestines to be displaced far forward in the body cavity and to be void of food. The entire body cavity is occupied by the enlargened gonads.

During October and November, serum cholesterol and triglycerides make up only 50% of the total lipids, the remaining total lipids are relected in phospholipids and lipoproteins (Miller, 1971). In females the major phospholipoprotein is vitellogenin (Barnes and Blackstone, 1973). The synthesis and movement of this product is stimulated by estrogen action and the gonadotropins (Crim and Idler, 1978; Lambert et al, 1978). In males the phospholipids are used for development of testes and seminal fluids (Schreck, 1974). This study did not encompass the monitoring of phospholipids nor lipoproteins. It is felt that further research is needed in better understanding the roles of these two substances in migration and spawning.

This study looked at condition factor, an index of fatness in fish. According to Royce (1972) increasing "K" indicates weight increase while the linear proportions stay constant. This study showed very little change in "K" from May to December. The small drop seen in November by the females (Fig. 4) may reflect a transfer of fats from body storage to the gonads (Schreck, 1974, Woodhead, 1975). Royce (1959) indicates that variation in "K" within a single population and that variation in "K" results from normal seasonal fluctuations in their metabolic balance. These

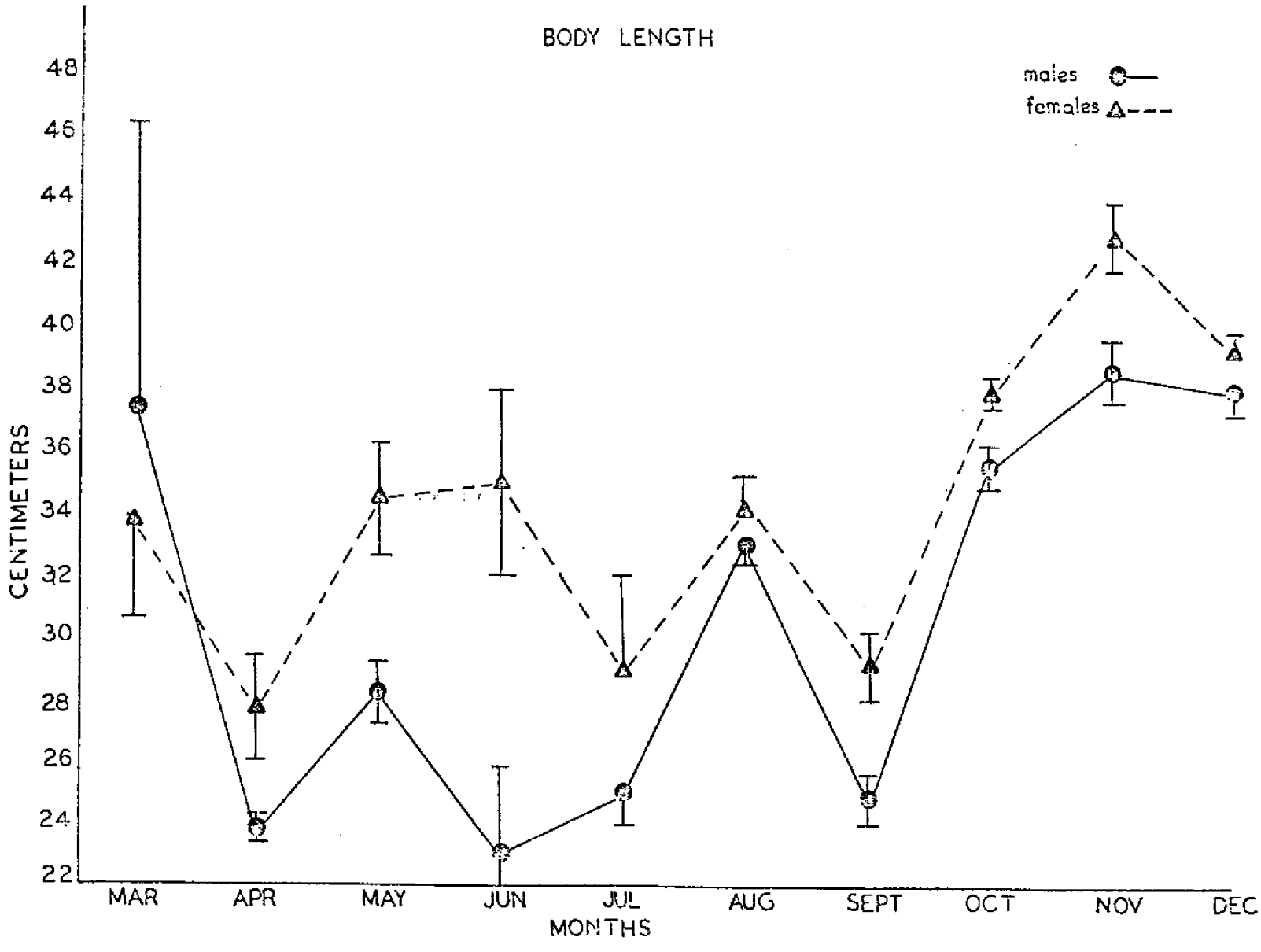
changes may be coincidental with maturation and subsequent release of reproductive products.

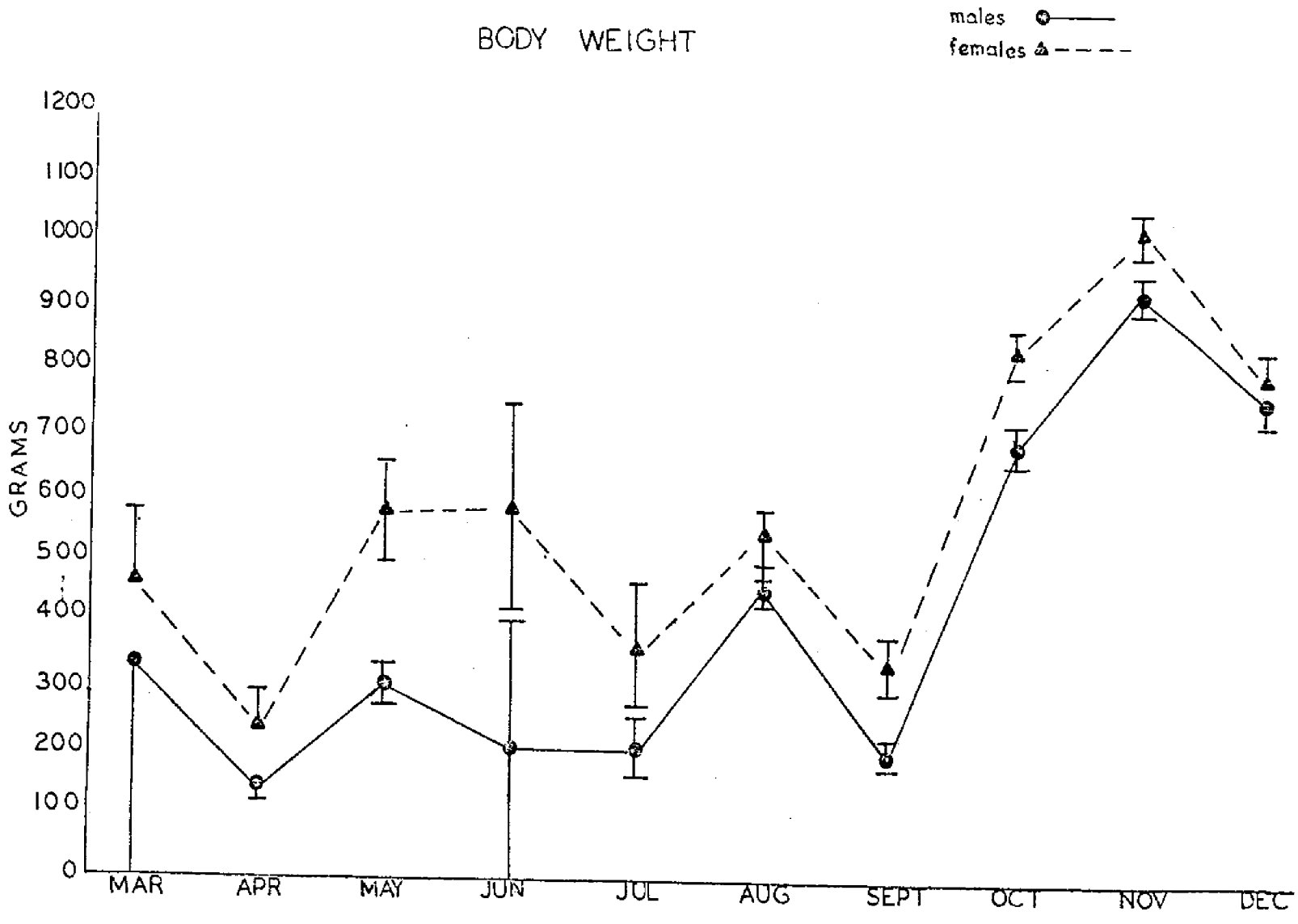
SUMMARY

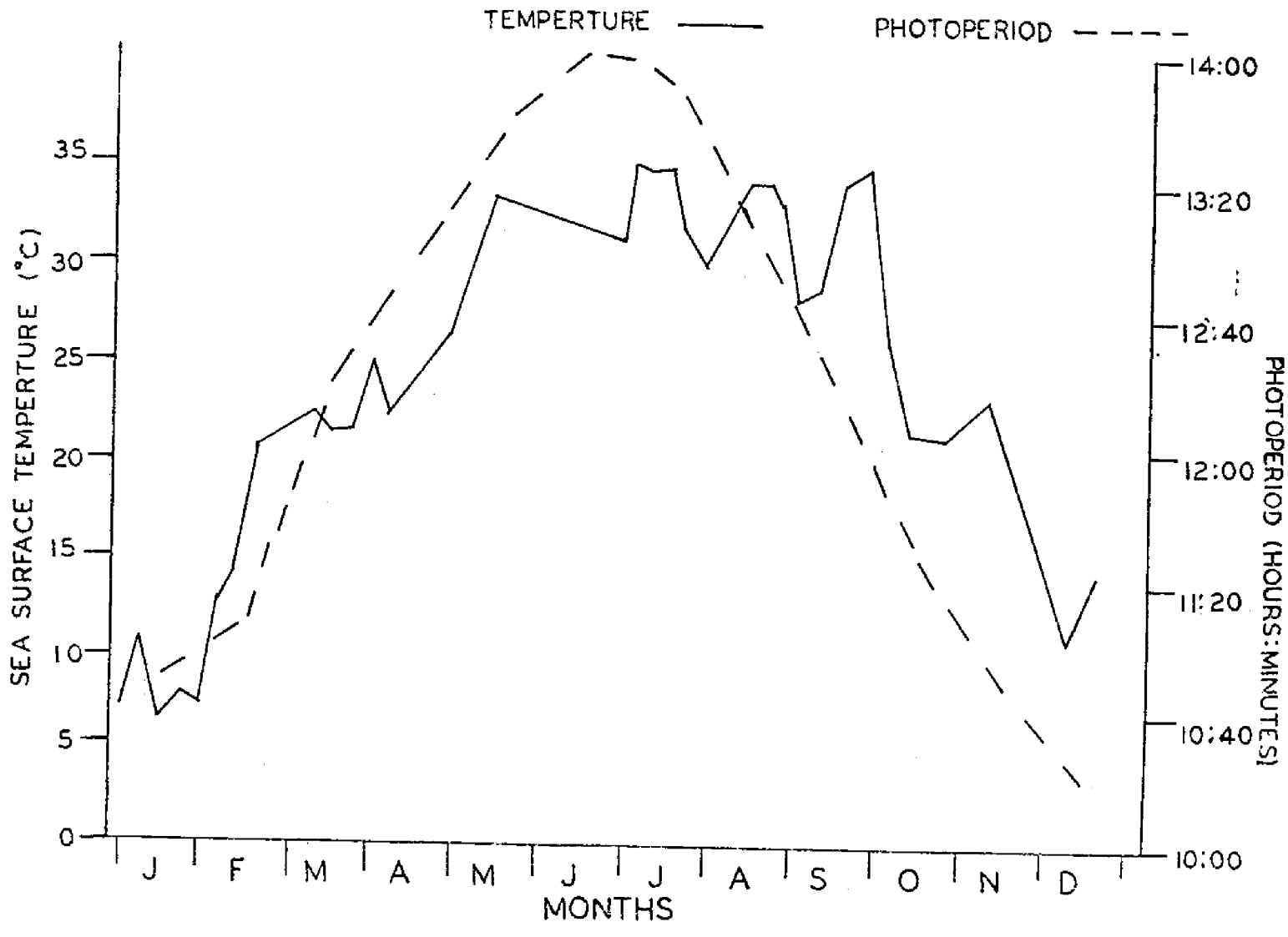
The striped mullet, Mugil cephalus, undergoes a lengthy annual migration to spawn. The fish has to meet major energy requirements of migration and developing gonads without feeding along the migration. This study has shown that serum lipids play an inportant role in transporting the energy needed for the fall migration and spawn.

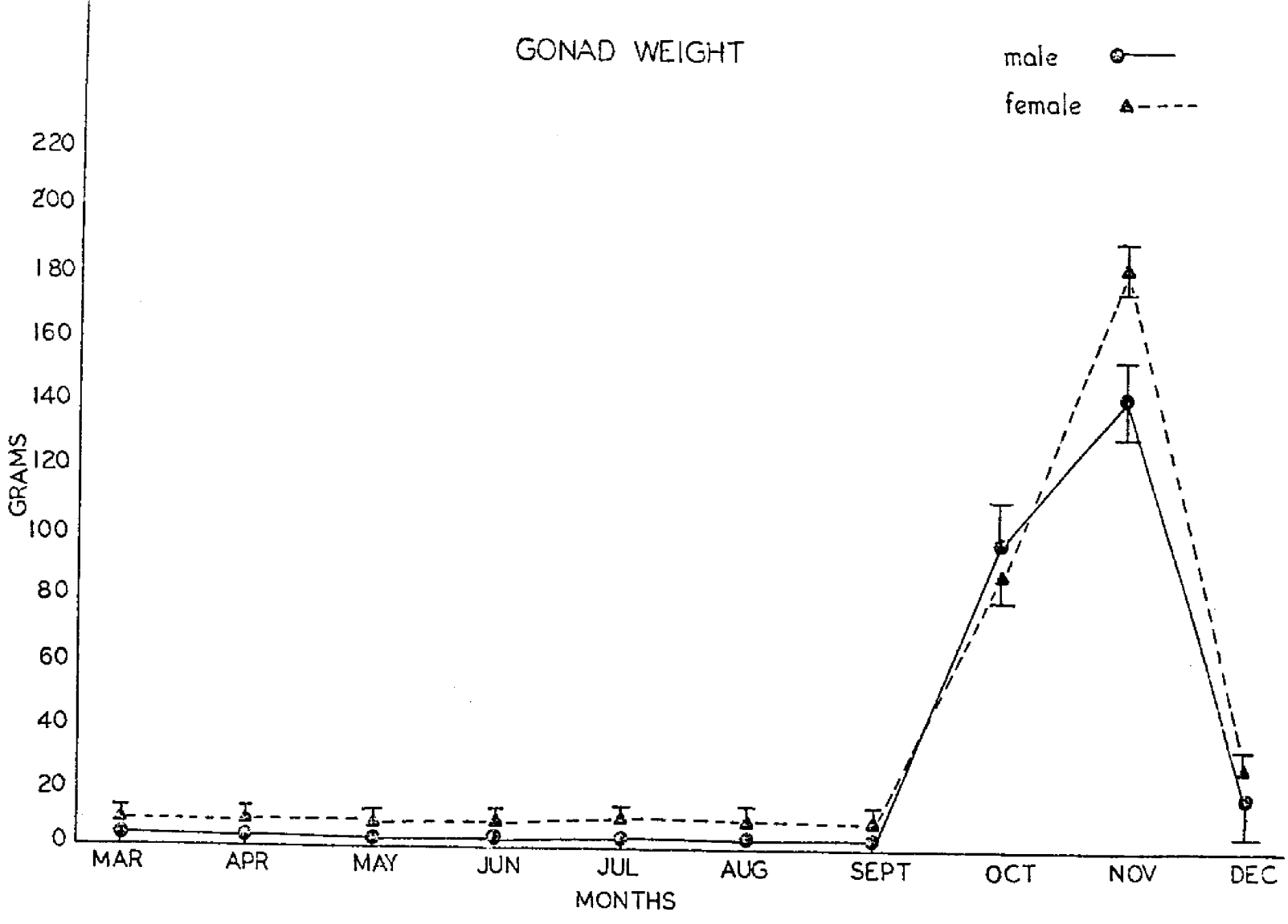
Utilizing radioimmunoassay techniques for monitoring the gonadal steriods, this study has shown a substantial seasonal fluctuation which is reflective of the fall migrations and spawning of the striped mullet. This data inconjunction with gonad weight and GSI changes indicates an October-November spawning period with adults returning to the estuary in late December, which substantiates other researchers findings.

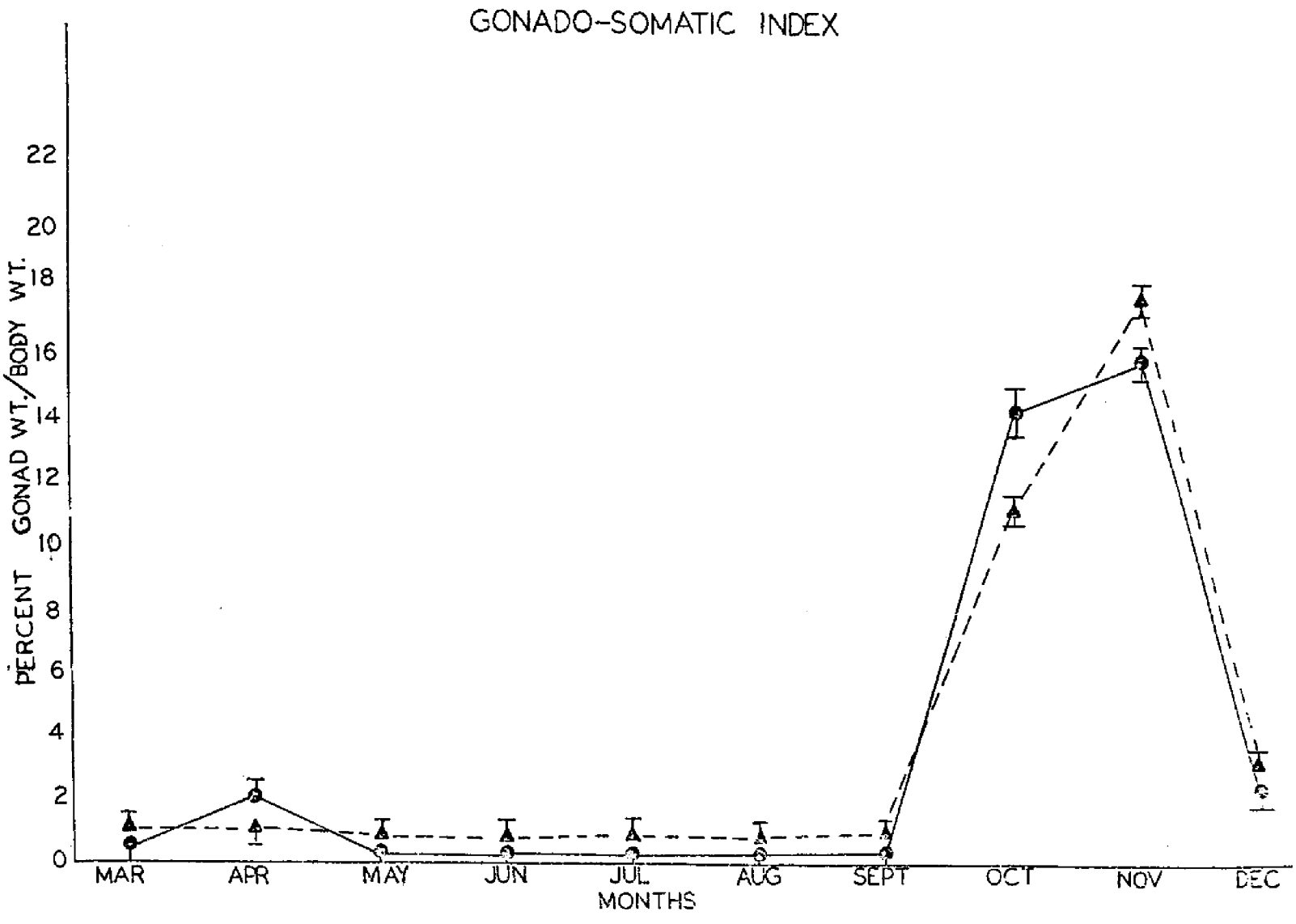
The use of radioimmunoassay techniques inconjunction with other sexual maturity factors may prove to be a valuable asset in maintaining stocks of other valuable commercial fish.

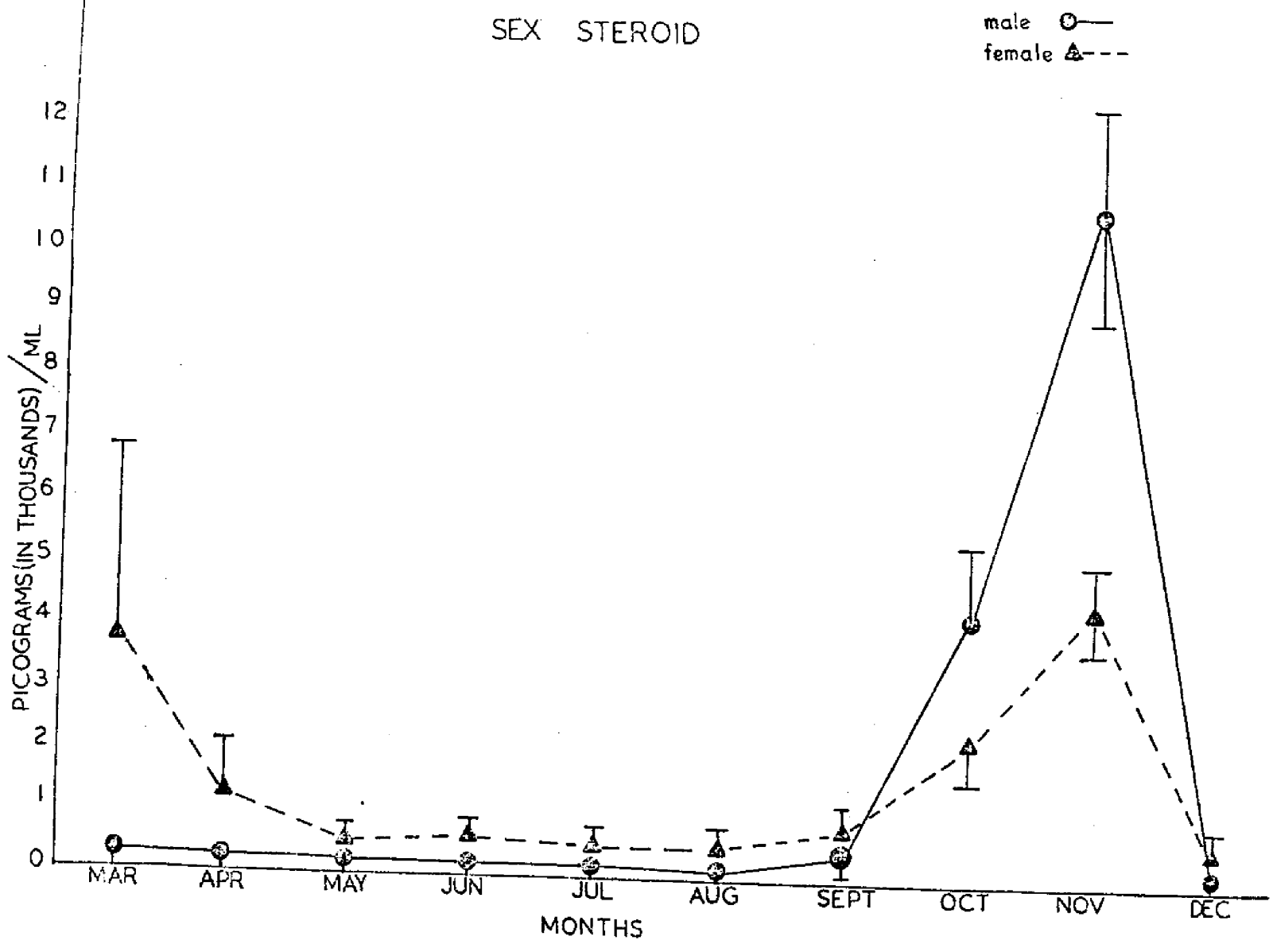

BODY LENGTH
males
females
CENTIMETERS
48
46
44
42
40
38
36
34
32
30
28
26
24
22
MAR
APR
MAY
JUN
JUL
AUG
SEPT
OCT
NOV
DEC
MONTHS

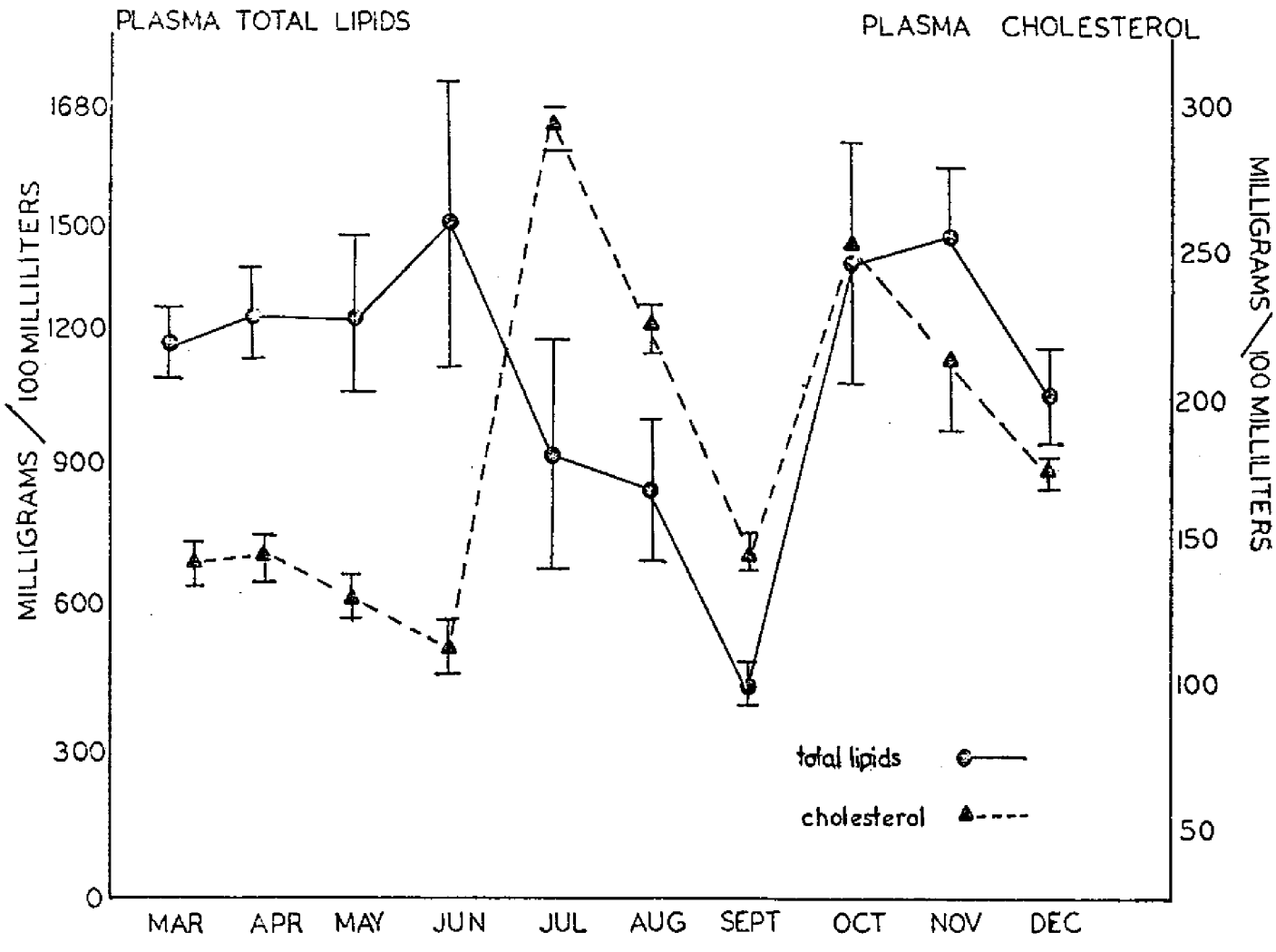

BODY WEIGHT
males
females
GRAMS
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
MAR
APR
MAY
JUN
JUL
AUG
SEPT
OCT
NOV
DEC

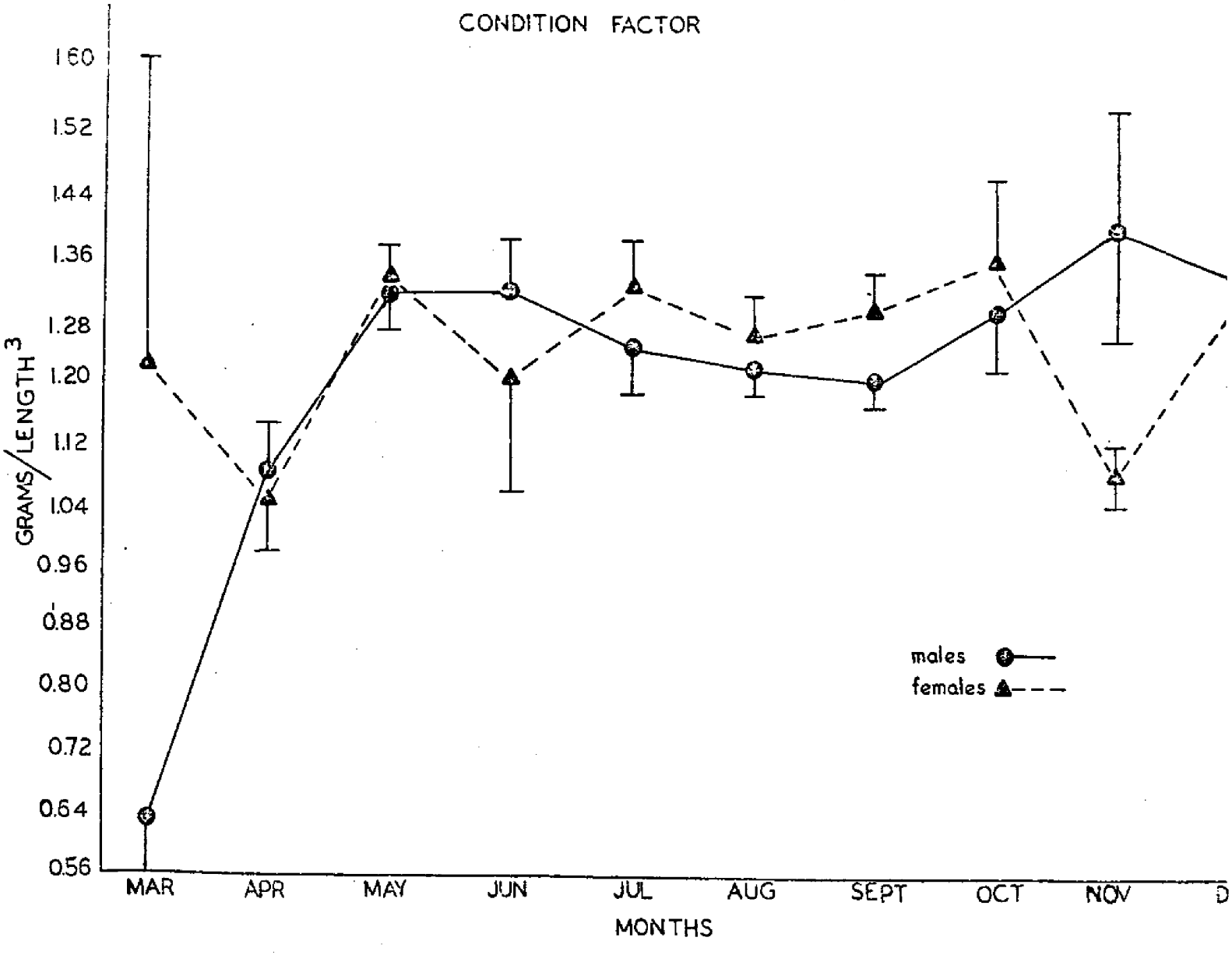

TEMPERTURE
PHOTOPERIOD
SEA SURFACE TEMPERTURE (°C)
0
5
10
15
20
25
30
35
14:00
13:20
12:40
12:00
11:20
10:40
10:00
PHOTOPERIOD (HOURS:MINUTES)
J
F
M
A
M
J
J
A
S
O
N
D
MONTHS


GONAD WEIGHT
male
female
220
200
180
160
140
120
100
80
60
40
20
0
GRAMS
MAR
APR
MAY
JUN
JUL
AUG
SEPT
OCT
NOV
DEC
MONTHS


GONADO-SOMATIC INDEX
PERCENT GONAD WT./BODY WT.
22
20
18
16
14
12
10
8
6
4
2
0
MAR
APR
MAY
JUN
JUL
AUG
SEPT
OCT
NOV
DEC
MONTHS


SEX STEROID
male
female
PICOGRAMS (IN THOUSANDS) / ML
0
1
2
3
4
5
6
7
8
9
10
11
12
MAR
APR
MAY
JUN
JUL
AUG
SEPT
OCT
NOV
DEC
MONTHS


PLASMA TOTAL LIPIDS
PLASMA CHOLESTEROL
MILLIGRAMS / 100 MILLILITERS
MILLIGRAMS / 100 MILLILITERS
1680
1500
1200
900
600
300
0
300
250
200
150
100
50
MAR
APR
MAY
JUN
JUL
AUG
SEPT
OCT
NOV
DEC
total lipids
cholesterol


CONDITION FACTOR
GRAMS/LENGTH 3
1.60
1.52
1.44
1.36
1.28
1.20
1.12
1.04
0.96
0.88
0.80
0.72
0.64
0.56
MAR
APR
MAY
JUN
JUL
AUG
SEPT
OCT
NOV
D
MONTHS
males
females