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**RESEARCH ARTICLE**

# Use of nematode maturity index for the determination of ecological quality status: a case study from the Black Sea

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## Abstract

Free-living marine nematodes inhabiting the shallow waters (3m) of Sinop Bay were analyzed to evaluate their usage as biological indicators. Their functional diversity was studied seasonally (August 2009, October 2009, January 2010 and April 2010). Life history strategies (c–p scaling) of nematode assemblages were examined. The Maturity Index (MI) based on c–p scale was calculated to test if it may be used for the interpretation of the environmental conditions and the determination of the ecological quality status of benthic habitats in the Black Sea ecosystem. Highest MI value was found at station C1 in April where the lowest organic matter concentration was recorded. The results indicated a possible utilization of MI and c–p class percentages to identify the ecological quality status of benthic environments according to Water Framework Directive.

**Keywords**: Free-living marine nematodes, Water Framework Directive, Maturity Index, functional diversity, Sinop Bay

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## Introduction

Anthropogenic impact continuously grows on marine environment. The European Water Framework Directive (WFD, Directive 2000/60/EC) proposed the Ecological Quality Status (EQS) to understand the quality of waters. Biological indicators are regarded more meaningful for the assessment of ecological quality when compared to physico-chemical or abiotic variables alone (Femprucci and Balsamo 2012). Several biotic indices were developed based on data of macrobenthic organisms such as Ambi, Bentix, and Bopa. These indices have been utilized to classify coastal waters into five groups of ecological quality status (High, Good, Moderate, Poor, Bad).

Macrobenthic invertebrate species have been considered as good indicators of pollution due to their sedentary and relatively long lives and their tolerance to stress. However, meiofauna, particularly nematodes, have advantages compared to macrofauna due to their high species richness and high abundance, although the difficulty of nematode identification limits the use of such incredible group of organisms in ecological studies. Studies comparing meiofauna with macrofauna have revealed that meiofauna is more responsive to initial impacts of disturbance (Schratzberger *et al.* 2000; Whomersley *et al.* 2009) On the other hand, the vast number of free-living marine nematodes encountered in small meiobenthic samples from almost all kinds of habitats can make them a suitable group of organisms to be used in environmental studies. Also the fact that they live in close contact with sediment (consequently with contaminants) and spend their whole life as benthic organisms means the changes in environment are reflected in their faunal analysis (Vranken and Heip 1986). Most nematode species have direct larval phase without a temporary pelagic phase and have short life cycles of a few months. Hence, short term studies can readily reveal changes in their community structure (Liu 2009). Free-living nematodes are the most diverse and numerically abundant organisms in aquatic ecosystems and they are among the tolerant taxa which live in polluted habitats. Distribution of nematodes has been found to have relations with granulometric structure of the sediment (Vincx *et al.* 1990; Vanaverbeke *et al.* 2002). A recent study points out the relation between the diversity of nematodes and ecosystem functioning (Danovaro *et al.* 2008). From these points of view, nematodes have been proposed as an indicator for assessing marine ecological quality within the Water Framework Directive (Moreno *et al.* 2011).

Nematodes are generally analysed based on their taxonomic diversity, however, Maturity Index (MI) is based on the reproductive and life history strategies of nematodes and not widely used for marine nematodes (Bongers 1990). It is a tool used for years in study of terrestrial and freshwater habitats to indicate the environmental quality based on the nematode community, and also applicable to marine and brackish water ecosystems (Bongers *et al.* 1991). Disturbed environments are represented by low MI values, and more pristine environments are indicated by higher MI values. Use of the diversity of free-living marine nematodes in monitoring of marine ecosystems is emphasized in recent papers (Moreno *et al.* 2008; Semprucci *et al.* 2008; Moreno *et al.* 2011; Semprucci and Balsamo 2012; Balsamo *et al.* 2012; Semprucci *et al.* 2013). The main purpose of this study is to apply the MI and c–p values for the first time to marine nematode data of a region in the Black Sea (Sinop Bay, Turkey) and to see if it works for the determination of ecological quality status as proposed in the Marine Strategy Directive.

## Materials and Methods

Sampling area was located at the central Turkish Black Sea, Sinop Bay (Figure 1). Station characteristics are given in Table 1. Undisturbed sediment material was collected monthly by Scuba divers using a metal sediment corer of 4 cm diameter (sampling area 12.56 cm$^2$) positioned into a 20x20 quadrate to a depth of 10 cm at four stations (3 m) during the period between August 2009 and July 2010. An extra sediment sample was taken from each station for sediment parameters and stored at -21°C until measurements were made. Surface water parameters were obtained *in situ* (Table 2).

**Table 1.** Station characteristics

| Stations | Depth | Latitude | Longitude |
|---|---|---|---|
| A1 | 3 m | 42°00'58" | 35°11'41" |
| B1 | 3 m | 42°01'02" | 35°10'58" |
| C1 | 3 m | 42°01'06" | 35°08'07" |
| D1 | 3 m | 42°00'09" | 35°06'58" |

**Table 2.** Environmental parameters of surface water and sediment of the stations in each season (DO: dissolved oxygen, Temp.: temperature, Sal.: salinity, OM: organic matter)

| Stations | pH | DO mg/L | Temp. (°C) | Sal. (ppt) | OM (%) |
|---|---|---|---|---|---|
| A1-Aug | 7,72 | 6,8 | 23,23 | 17,52 | 0,81 |
| A1-Oct | 7,94 | 7,8 | 20,88 | 17,67 | 0,80 |
| A1-Jan | 7,02 | 3,39 | 9,55 | 17,74 | 0,30 |
| A1-Apr | 7,42 | 3,36 | 13,06 | 17,56 | 0,13 |
| B1-Aug | 7,12 | 5,98 | 23,48 | 17,73 | 0,05 |
| B1-Oct | 7,54 | 7,8 | 20,76 | 17,67 | 0,05 |
| B1-Jan | 7,12 | 5,6 | 9,5 | 17,68 | 0,08 |
| B1-Apr | 7,12 | 3,41 | 12,84 | 17,65 | 0,03 |
| C1-Aug | 8,61 | 5,9 | 23,64 | 17,87 | 0,71 |
| C1-Oct | 8,26 | 7,5 | 20,53 | 17,65 | 0,06 |
| C1-Jan | 8,02 | 5,95 | 9,51 | 17,7 | 0,03 |
| C1-Apr | 8,19 | 3,84 | 12,59 | 17,65 | 0,02 |
| D1-Aug | 9,12 | 5,84 | 23,55 | 17,88 | 0,32 |
| D1-Oct | 8,39 | 7,1 | 20,56 | 17,69 | 0,13 |
| D1-Jan | 7,56 | 5,74 | 9,16 | 17,69 | 0,07 |
| D1-Apr | 7,62 | 4,4 | 12,21 | 17,71 | 0,09 |

The samples were fixed onboard with 75% ethanol. One replicate of samples obtained from sandy and muddy soft bottoms at the four shallow stations with a depth of 3 m was evaluated for the present study. Data of four seasons were taken into consideration summing up to sixteen occasions. In the laboratory, the fauna of macro- and meiobenthos was separated by wet sieving. Specimens retained on 64 µm mesh size were extracted and counted by the help of modified Bogorov chambers. Nematodes were mounted on glass slides with glycerine+water solution, identified up to genus level following Platt and Warwick (1983, 1988) and Warwick *et al.* (1998). Nematode genera are classified into five ecological groups (1 to 5), ranging from extreme *r*-strategists (colonisers) to extreme *k*-strategists (persisters) according to Bongers *et al.* (1991). "Coloniser" nematodes at the lower end of the c–p scale are considered opportunists and therefore indicate the availability of food; 'persister' nematodes at the high end of the scale indicate that there is a stable system and food web is complex. Extreme colonisers or in other words enrichment opportunistics (c–p1) known from marine or brackish environments are composed of only several members of rhabditids and diplogasterids (Bouwman 1983).

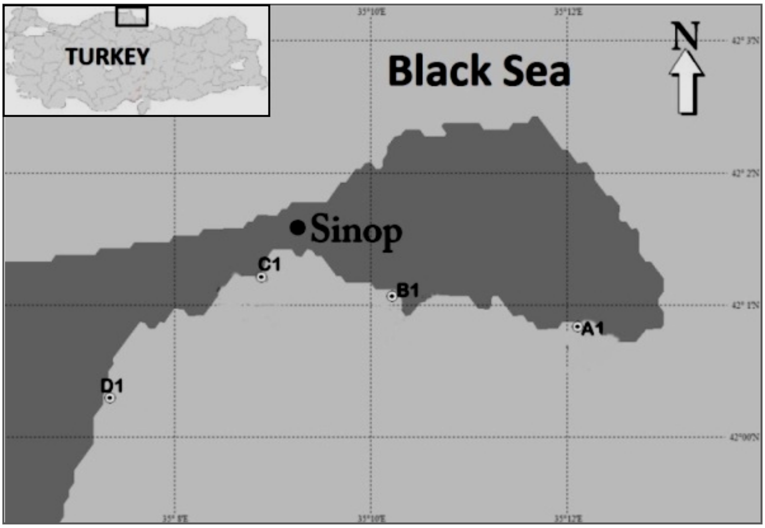

**Figure 1.** Study area showing the location of the sampling stations

Group c–p1 has generation times of only some days, high colonization ability, and tolerance to environmental stress. They have a high metabolic activity. Their population growth under conditions with rich food is explosive. Nematodes assigned to c–p2 have a short generation time, they respond more slowly to environmental enrichment than c–p1 nematodes but increase in abundance under stressed conditions. They occur in all environments, and very tolerant of pollutants and other disturbances (Herris and Bongers 2009). Group c–p3 is an intermediate class, has longer generation time than the previous class and includes some Chromadoridae (recalibration made at genus level) and relatively sensitive to disturbances. Nematodes assigned to the group c–p4 are characterized by a long generation time, permeable cuticle and recognized as highly sensitive to stress and pollutants. Extreme persisters are composed of larger omnivores and predators as Enoplidae and Leptosomatidae (c–p5). They present with a generation time of one year, low colonisation ability, low reproduction rates and they have a permeable cuticle, are very sensitive to pollutants and other disturbances in marine meiobenthos than are *r*-strategists (Warwick 1986).

If an assemblage is exposed to pollution, colonisers are more tolerant than persisters. If *k*-strategists disappear their resources will then serve as food for more tolerant species, resulting in increase of the number of colonisers under disturbed conditions (Bongers *et al.* 1991, 1995; Bongers and Bongers 1998). The classification of marine free-living nematodes based on c—p scale was made considering their body size, generation time, dominance in samples and sensitivity to pollution (Bongers 1999).

The MI (Bongers 1990; Bongers *et al.* 1991) was calculated as the weigthed mean of the genera scores. Each group of nematodes (c—p values) based on their life histories were used for the calculation.

$$\text{Maturity Index (MI)} = \sum_{i=1}^{n} v(i).\ f(i)$$

Where $v(i)$ is the c—p score of genus i and $f(i)$ is the frequency of that genus in a sample.

Genus *Monhystera* was originally assigned to the class c—p1, however it has later been given a c—p value of 2 (De Goede *et al.* 1993; Bongers *et al.* 1995), due to the reason that it is a general opportunist.

Moreno *et al.* (2011) suggested thresholds based on metadata obtained from several areas of the Mediterranean Sea. These thresholds are based on MI values and c—p % indicating the five ecological quality status of the considered area (Table 3). Our stations have been evaluated according to the values given below.

**Table 3.** Proposed thresholds based on marine nematode data by Moreno *et al.* (2011) for the determination of ecological quality status of a given environment

| **Indicator** | **High** | **Good** | **Moderate** | **Poor** | **Bad** |
|---|---|---|---|---|---|
| **MI** | >2.8 | 2.8≤MI<2.6 | 2.6≤MI<2.4 | 2.4≤MI<2.2 | ≤2.2 |
| **c—p** | c—p2 ≤ 50%<br>c—p4 >10% | c—p 2 ≥ 50%<br>c—p 4 > 10% | c—p 2 ≥ 50%<br>3 < c—p 4 < 10% | c—p 2 > 60%<br>c—p 4 < 3% | c—p 2 > 80% |
| **Sensitive/ Tolerant Genera (>10%)** | *Microlaimus,*<br>*Richtersia,*<br>*Oncholaimus,*<br>*Pomponema,*<br>*Epacanthion,*<br>Desmoscolecidae | *Setosabatieria,*<br>*Ptycholaimellus,*<br>*Halalaimus* | *Anticoma,*<br>*Desmodora,*<br>*Spirinia,*<br>*Marylynia,*<br>*Prochromadorella* | *Daptonema / Theristus,*<br>*Paralongicyatholaimus,*<br>*Parodontophora,*<br>*Odontophora* | *Paracomesoma,*<br>*Terschellingia,*<br>*Sabatieria* |

## Results and Discussion

Regarding c—p values of the stations, c—p1 and c—p5 were absent in the studied samples. Group c—p2 was dominant in eight of 16 occasions as seen in Table 4 (four months, four seasons). The members of this class are known to be opportunistic and they increase in abundance under disturbed conditions (Bongers and Bongers 1998). St. D1-Oct and D1-Apr revealed group c—p2 with a dominance of 100%. St C1-Oct and St B1-Aug revealed high abundances of c—p2 (71% and 60%, respectively). Group c—p3 was found to be most abundant at St. C1-Apr (71%) and A1-Aug (67%). St. B1-Jan, B1-Apr and D1-Jan also showed 50% abundance of group c—p3. St. A1-Apr was the only location where the group c—p4 (33%) was recorded which suggested better conditions than other stations.

**Table 4.** Dominant colonizer-persister classes at the stations and the calculated Maturity Index values based on the c—p scales

| Stations | Maturity Index | Dominant c—p class (%) |
|---|---|---|
| A1-Aug | 2.55 | c—p3 (67) |
| A1-Oct | 2.76 | c—p2 (40) |
| A1-Jan | 2.63 | c—p2 (50) |
| A1-Apr | 3.19 | c—p2, c—p3, c—p4 (33) |
| B1-Aug | 2.27 | c—p2 (60) |
| B1-Oct | 2.64 | c—p2 (50) |
| B1-Jan | 2.68 | c—p3 (50) |
| B1-Apr | 2.81 | c—p3 (50) |
| C1-Aug | 2.41 | c—p3 (47) |
| C1-Oct | 0.46 | c—p2 (71) |
| C1-Jan | 2.61 | c—p2 (54) |
| C1-Apr | 3.33 | c—p3 (71) |
| D1-Aug | 0.00 | - |
| D1-Oct | 2.00 | c—p2 (100) |
| D1-Jan | 2.50 | c—p2,c—p3 (50) |
| D1-Apr | 2.00 | c—p2 (100) |

The MI values ranged from 0.44 (St C1, October 2009) to 3.33 (St C1, April 2010) excluding 0.00 calculated for St D1, August 2009 due to the reason that no individual was recorded. MI reached its highest values at stations A1, B1 and C1 in April. The most stable station with an MI value of 3.33 (C1-Apr) presented also with the lowest organic matter content (0.02 %). As seen in Figure 2, St B1 showed low OM% and good MI values. St A1-Apr revealed a high MI value (3.19) and it was in agreement with low OM% (0.13). Excluding St D1, the lowest MI value (0.44) was estimated for station C2 in October with dominance of the group c—p2 (57%). When the temporal trends were taken into consideration, a general consistency was observed and higher MI values were recorded usually when lower organic matter percentages were found.

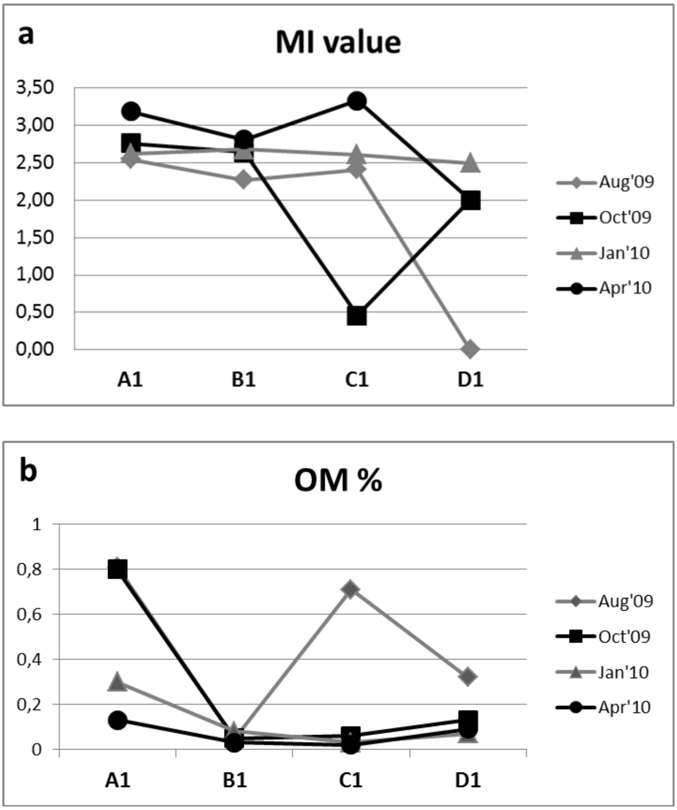

**Figure 2.** Spatial changes of a) Maturity Index (MI) values and b) Organic matter (OM) percentages at four stations

Ecological status of our sampling stations were estimated depending on MI and c─p values, also sensitive/tolerant genera were taken into consideration. Coinciding with MI results, St A1-Apr, St B1-Apr, St C1-Apr were found to be at High Ecological Quality Status (EQS) depending on c─p values. When the sampling sites assigned to High EQS were considered, group c─p3 was dominant at St B1-Apr (50%) and St C1-Apr (71%) while in same proportions at St A1-Apr. St A1-Jan was found to be at Good ECQ, St C1-Aug at Moderate EQS, St D1-Oct at Bad EQS when both MI and c─p values were regarded.

Several different results were also recorded although they were not conflicting. According to c─p values, St A1-Oct and St B1-Oct were at high quality, however considering MI values, these stations were at good quality. St C1-Aug was assigned to high ecological quality status by its c─p values, whereas it was classified at moderate quality depending on its MI value (Table 5).

**Table 5**. Ecological quality status of our sampling stations in Sinop Bay classified according to Maturity Index (MI) and c—p values

| **Indicator** | **High** | **Good** | **Moderate** | **Poor** | **Bad** |
|---|---|---|---|---|---|
| Sinop Bay | | | | | |
| MI | A1-Apr,<br>B1-Apr,<br>C1-Apr | A1-Jan,<br>B1-Jan,<br>C1-Jan,<br>A1-Oct,<br>B1-Oct | A1-Aug,<br>C1-Aug | B1-Aug | C1-Oct,<br>D1-Oct |
| c—p | A1-Apr, B1-Apr,<br>C1-Apr, A1-Oct,<br>B1-Oct, C1-Aug | A1-Jan,<br>B1-Aug | C1-Jan | | D1-Apr,<br>D1-Oct |
| Sensitive/Tolerant genera | C1-Aug,<br>A1-Aug,<br>A1-Oct | | C1-Aug,<br>C1-Apr | A1-Aug,<br>A1-Oct,<br>C1-Aug | A1-Aug |

Bongers *et al.* (1991) applied MI to Lambshead (1986)'s dataset and suggested that MI decreased by pollution and increased during the colonization process of the nematodes. MI for clean stations ranged between 2.31 and 2.75 whereas for polluted stations the values were lower, ranging between 2.03 and 2.21. Seasonal fluctuations were recorded for MI values when the nematode fauna was analyzed after Boucher (1980) by Bongers *et al.* (1991). For the purpose to use nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems, Moreno *et al.* (2011) evaluated the use of nematodes as biological indicators of environmental quality in accordance with WFD and suggested several thresholds for the determination of ecological quality status of a given environment as a result of a meta-analysis for the Mediterranean. The suggestion has also been supported with the presence/absence of specific nematode genera indicating the five ecological quality classes. MI and colonizer-persister class percentage (c—p %) were concluded to give better results with genus level calculations compared to the other widely used indices. Semprucci *et al.* (2010) proposed that MI could be a good descriptor of stress, because it is influenced to a lesser extent by natural environmental variables (e.g. sediment granulometry) than other available indices (i.e. H' and J). Our results highlighted the compatible results of MI and c—p values for the assignment of sampling sites to five ecological quality classes. Values are meaningful when organic matter contents (%) of sites are considered. The highest MI (3.33) was calculated for Station C1 in April, for which the lowest organic matter % was recorded (0.02) among all other sampling cases. A1-Apr, B1-Apr and C1-Apr showed high ecological quality status according to MI tresholds. A1-Jan, B1-Jan and C1-Jan were classified as good quality based on their MI values (2.63, 2.63 and 2.61, respectively). Consistently, these stations were also classified to be in high and good ecological status according to AMBI results calculated based on the data of the macrozoobenthic invertebrates obtained at the same stations (Sezgin *et al.* 2013).

The evaluation of MI and c–p values in this study fitted to ecological quality classes, although several different assessments have been observed. We suggest that % c–p values gave better results in assigning the sites to five classes. The results of the present study generally revealed that the ecological quality of the investigated area varied from high to poor quality with seasonal fluctuations mainly based on touristic activities during summer, since the stations were located at coastal beach areas. In winter and spring the sampling stations seem to be at better conditions.

## Conclusion

MI has only recently been used to determine ecological quality status in Mediterranean ecosystems based on proposed thresholds (Moreno *et al.* 2011). This study was the first attempt for its use in the Black Sea which has unique characteristics and conditions. The results of our study indicated that the functional diversity, including c–p scale and MI of nematodes may give us clues about the environmental status of coastal areas. These indices may serve in monitoring coastal areas and determination of ecological quality status on the condition that no other confounding factors are available such as difference in granulometric structure and water depths. The reason is that the distribution and abundance of nematodes, particularly their trophic diversity, are critically affected by these environmental parameters. The c–p percentage values found to better fit in assigning the sampling sites to the five classes of the EQS. Moreover, suggested thresholds for MI may need to be modified according to a result of future studies on the Black Sea nematode assemblages, since they were originally determined for Mediterranean assemblages. Finally, according to WFD, development of new indices will be of significance to better understand the benthic ecosystem. This target has been accomplished for macrobenthos, however still at its initial stages for meiobenthos and marine free-living nematodes. If new indices can be applied based on taxonomic data, particularly at genus level, they can be candidates to serve in monitoring environmental quality which is a significant component of management of marine ecosystems. To this end, studies are needed also for the Black Sea on expanded datasets to compare the thresholds with those of the Mediterranean and to further develop the use of nematode diversity in evaluating ecological quality status of specific environments.

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## Nematod maturity indeksinin ekolojik kalite durumunun belirlenmesinde kullanımı: Karadeniz'den bir olgu çalışması

### Özet

Sinop Körfezi'nin sığ sularında (3 m) serbest yaşayan denizel nematodlar, biyolojik indikatör olarak kullanımlarının değerlendirilmeleri amacıyla analiz edilmiştir. Ağustos 2009, Ekim 2009, Ocak 2010 ve Nisan 2010 örneklemelerinde elde edilen veriler üzerinden nematodların fonksiyonel çeşitlilikleri mevsimsel olarak incelenmiştir. Nematod topluluklarının yaşam biçimi stratejileri (c—p skalası) analiz edilmiştir. C—p sınıflandırmasına dayalı olarak Maturity İndeksi hesaplanmış ve bu indeksin çevresel koşulların yorumlanması ve istasyonların ekolojik kalite durumlarının belirlenmesi için Karadeniz'de kullanıma uygun olup olmadığı test edilmiştir. En yüksek Maturity İndeks değeri Nisan ayında C1 istasyonunda tespit edilmiştir ve bu istasyonda aynı zamanda en düşük organik madde yüzdesi kaydedilmiştir. Sonuçlar, Maturity indeksinin ve c—p yüzdelerinin, Su Çerçeve Direktifine uygun olarak ekolojik kalite durumunu gösteren beş sınıfın belirlenmesinde kullanılmasının mümkün olduğunu göstermiştir. Bununla birlikte, % c—p değerlerinin daha doğru bir sınıflandırma sağladığı görülmüştür. Bundan sonra daha kapsamlı veri kümeleriyle yapılacak Maturity indeks uygulamaları ve eşik değerlerinin revize edilmesi, indeksin Karadeniz'de daha sağlıklı çalışmasına katkıda bulunabilir.

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